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GLOMERULAR LESIONS ASSOCIATED WITH ENDOCARDITIS *

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Since Löhlein's publication in 1910,[1] it has been known that focal glomerular lesions may occur in the course of subacute bacterial endocarditis. The purpose of this investigation is to study in detail the pathogenesis of the focal lesions and also to describe other glomerular lesions associated with endocarditis that have heretofore received little or no attention.

The material available for study consisted chiefly of small pieces of kidneys preserved in formalin or tissues embedded in paraffin. Paraffin sections were cut and stained with hematoxylin and eosin, and azocarmine. Tissues preserved for ten years or longer in formalin stain fairly well with azocarmine if they are given a preliminary treatment with ammonia water and are refixed in Zenker's or Helly's fluid. Material secured fresh during the course of this study was usually fixed in Helly's fluid. The best results are obtained when fixation is accomplished by injection of the fixing fluid into the renal artery. In calculating the percentage of the various types of glomerular lesions, 100 or more glomeruli were examined. In most instances, however, only one block of tissue was available and it is recognized that this is a possible source of error. When the percentage of involved glomeruli is high, embolic lesions are found in all sections examined, but when the percentage is low, some sections show no embolic lesions. It is therefore possible that the study of numerous sections from various parts of the kidneys would have revealed some embolic lesions in the group that are recorded as negative.

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For purposes of this study endocarditis has been classified as follows:

I. Rheumatic endocarditis
II. Bacterial endocarditis
    A. Primary bacterial endocarditis
        (*a*) Acute
        (*b*) Subacute
    B. Secondary bacterial endocarditis

Rheumatic endocarditis is defined anatomically as the type in which the vegetations are all small and firm and incapable of forming emboli. The diagnosis is based entirely on the structure of the vegetations. No case was excluded because of failure to demonstrate Aschoff bodies in the myocardium. Bacterial endocarditis includes all instances in which the vegetations are soft and friable. Cases in which lesions of both types are present have been classified as bacterial endocarditis.

Only active rheumatic endocarditis has been studied. This is regarded as acute when there is no scarring of the leaflets, and recurrent when the leaflets show fibrous thickening from a former infection. Old healed rheumatic valves, that is, those without fresh vegetations, have been excluded from this study.

Bacterial endocarditis is divided into a "primary" type which seems to begin as a bacteremia, and a "secondary" type in which the invasion of the blood stream is obviously secondary to a previously established infection such as acute endometritis.

The distinction between acute and subacute endocarditis is arbitrary. Thayer [2] grouped all cases with a duration of less than one month as acute, but we have used a duration of six weeks as an arbitrary line of separation. The duration indicated in the tables is the estimated period in which the infective process was present, as indicated by chills, fever, positive blood cultures, enlargement of the spleen, embolic processes, and so on. But when a patient with cardiac decompensation from an old valvular defect develops acute endocarditis it is frequently impossible to determine the time of onset of the infectious process unless a thorough clinical study has been made. For this reason there is, no doubt, some error in the separation of the acute and subacute groups.

It would have been better to subdivide bacterial endocarditis still farther on the basis of etiology, as streptococcic, pneumococcic,

staphylococcic, gonococcic and influenzal, but the bacterial studies of our material are too meager to be of much value in this direction. Various investigations indicate that streptococci are responsible for nearly all cases of subacute endocarditis and a large proportion of the acute cases. There is no evidence that the streptococci found in the acute type are different from those in the subacute form. Endocarditis, caused by organisms other than streptococci, is nearly always of acute type, but there are occasional exceptions. Acute and subacute endocarditis cannot be separated satisfactorily on an etiological basis.

I. Rheumatic Endocarditis

The kidneys from 104 cases of active rheumatic endocarditis have been examined. In every instance fresh rheumatic vegetations were found on the valve leaflets. In 53 cases there was no evidence of old valvular disease, and the lesion was therefore regarded as acute. In 51 cases the lesion was regarded as a recurrent acute infection because of the presence of old scar tissue in the leaflets. There is no significant difference in the incidence of glomerular lesions in the acute and recurrent forms. In 81 of the 104 cases (77.8 per cent) the glomeruli are either entirely normal or they show only a slight alteration. In one instance a typical advanced chronic diffuse glomerulonephritis was present. In the remaining 22 cases (21 per cent) the glomeruli show a moderate but definite acute diffuse inflammatory reaction. Nearly all of the glomeruli are involved. There is a definite increase in the number of endothelial cells and their cytoplasm has become conspicuous. The capillary basement membrane is occasionally thickened. The appearance is such as is shown in Figure 1. Occasionally there is also a notable increase in the number of leukocytes in the glomerular capillaries.

The increase of endothelium is much less prominent and the capillary obstruction much less pronounced than in clinical acute glomerulonephritis. There are no intracapillary fibers but in other respects the lesion differs only in degree from the clinical case. It is probable, however, that lesions of this moderate degree readily return to normal and do not often progress to the extent of complete capillary obstruction.

In 2 of the 22 cases the endothelial proliferation is very prominent

TABLE I

Acute Bacterial Endocarditis *

Number	Age	Sex	Duration	Old valvular defect	Blood culture	Weight of spleen	Glomerular lesions		
							Infarcts	Embolic	Diffuse
	years					*gm.*			
14–209.....	42	M	1 wk.	+	o	325	+	–	+
15–391.....	43	M	3 wk.	–	o	153	–	–	–
16–54.....	35	M	2 wk.	–	o	762	+	–	–
16–128.....	19	F	5 days	+	o	350	–	–	–
17–31.....	19	M	3 wk.	+	s.h.	465	+	–	–
17–213.....	43	F	2 wk.	–	o	325	–	–	–
19–197.....	34	F	6 days	+	o	520	+	–	–
20–2.....	18	M	5 wk.	+	o	280	+	–	–
21–124.....	78	M	4 wk.	+	o	130	+	–	–
22–148.....	19	F	1 mo.	–	o	270	+	–	+
25–600.....	38	F	10 days	+	o	375	–	–	–
25–1022.....	41	F	1 mo.	+	o	?	+	–	–
26–600.....	2	F	1 mo.	–	s.v.	60	–	–	–
26–635.....	52	M	?	–	o	250	–	–	+
26–818.....	25	M	4 days	–	o	190	+	–	–
27–306.....	24	F	3 days	+	o	300	+	a	–
27–479.....	33	F	1 mo.	–	o	155	+	–	+
27–847.....	12	F	10 days	–	st.	55	–	–	–
27–1008.....	58	F	1 mo.	+	o	210	+	–	–
28–185.....	16	F	1 wk.	–	st.	400	+	a	–
28–186.....	39	M	2 days	+	s.	275	–	a	–
28–343.....	26	M	1 mo.	+	s.v.	420	+	–	+
28–481.....	18	M	5 wk.	–	o	550	–	–	–
28–520.....	57	M	5 days	+	o	310	+	+	–
28–1251.....	34	F	?	–	o	160	–	–	–
28–1276.....	55	M	?	–	o	215	–	–	+
28–1347.....	30	M	?	–	o	85	+	–	–
28–1348.....	64	M	3 wk.	–	s.v.	300	+	–	–
28–1628.....	21	M	5 wk.	–	s.v.	340	+	–	+
29–736.....	31	M	6 days	–	o	200	–	a	–
29–824.....	37	F	3 wk.	–	pn.	200	–	–	+
29–1229.....	24	F	1 mo.	–	–	340	+	–	+
29–1278.....	42	M	6 days	–	o	660	+	–	+
29–1307.....	26	M	10 days	–	s.h.	425	+	+	–
29–1368.....	55	F	1 mo.	+	o	140	+	–	–
29–1387.....	44	F	2 wk.	+	o	475	+	+	–
29–1406.....	29	F	3 wk.	–	o	100	+	–	–
29–1441.....	21	F	1 mo.	–	st.	350	+	–	–
29–1533.....	72	F	?	+	o	90	+	–	–
29–1574.....	46	M	1 mo.	–	o	94	+	–	+
29–1893.....	69	F	1 mo.	–	s.v.	290	–	–	–
30–85.....	52	F	?	+	o	225	–	–	–
30–226.....	27	M	5 wk.	+	o	325	–	–	–

* Under embolic lesions "a" indicates that glomerular abscesses were present. Under blood culture "o" indicates that no blood culture was made; s.h. *Streptococcus hemolyticus*; s.v. *Streptococcus viridans*; s. streptococcus of undetermined type; st. staphylococcus; pn. pneumococcus.

TABLE I (continued)

Number	Age	Sex	Duration	Old valvular defect	Blood culture	Weight of spleen	Glomerular lesions		
							Infarcts	Embolic	Diffuse
	years					*gm.*			
30–589.....	53	F	?	+	o	300	–	–	–
30–1465.....	12	F	1 wk.	+	o	450	–	–	+
30–1655.....	61	M	2 wk.	–	o	314	–	–	–
30–1776.....	17	F	?	–	o	275	+	–	–
31–99.....	3½	M	8 days	+	o	100	–	–	–
31–554.....	39	M	1 wk.	+	o	175	+	–	+
31–579.....	50	F	1 mo.	–	–	710	+	–	+
31–708.....	15	F	3 wk.	+	o	344	+	–	–
31–939.....	42	F	5 wk.	–	o	133	+	–	–
31–1032.....	45	M	?	+	o	400	+	–	+
31–1193.....	26	F	?	–	o	375	–	–	–
31–1369.....	30	M	?	+	s.v.	250	–	–	–
31–1762.....	51	M	3 wk.	–	–	685	+	+	+

but still less pronounced than in a clinical case of acute glomerulonephritis.

In 3 of the 22 cases with acute diffuse glomerulitis, lesions of embolic type were also found in the glomeruli. The number of glomeruli involved was 1, 10 and 25 per cent respectively in the 3 cases. The lesions were all intracapillary thromboses. These will be described later, since they are similar to the small fresh lesions of subacute bacterial endocarditis.

There is no evident relation between acute glomerulitis and associated terminal infections. It is just as frequent in those dead of cardiac decompensation as in those with terminal pneumonia, peritonitis, and so on.

II. BACTERIAL ENDOCARDITIS

A total of 233 cases has been studied, of which 164 were primary and 69 secondary. Of the 164 primary infections, 56 were classed as acute and 108 as subacute.

A. Primary Bacterial Endocarditis

(*a*) *Acute Cases:* The 56 cases of this group are listed in Table I. It will be noted that the duration of the infective process was not known accurately in 11 instances. Five of these patients had old

valvular defects with cardiac decompensation, and the presence of active endocarditis was not recognized clinically. In the other 6 cases the records are incomplete, but the duration was probably less than six weeks. Old valvular defects were present in 15 of the 56 cases (27 per cent). Blood cultures are recorded in only 16 cases. Of these, 6 showed *Streptococcus viridans*, 2 *Streptococcus hemolyticus*, 1 streptococcus of undetermined type, 3 staphylococcus, 1 pneumococcus, and 3 were negative.

The average weight of the spleen in 52 adults was 316 gm. The weight was 400 gm. or more in 13 instances. Infarction of the spleen or kidneys was present in 34 instances (61.7 per cent).

Glomerular lesions of embolic type were found in only 4 cases. In 3 of these, only small fresh intracapillary thromboses were found, 2 to 6 per cent of the glomeruli being involved. In the fourth case (29–1387), 3 per cent of the glomeruli showed small focal fibrous lesions, but no fresh lesions were found. The fibrous lesions may have resulted from an active endocarditis some time previous to the terminal attack, since an old valve defect was present.

There were 16 instances of acute diffuse glomerulitis, 2 of which were of exudative type and the others proliferative. Glomerular abscesses were found in 4 instances.

(*b*) *Subacute Cases:* (Table II.) Sixty-seven of the 108 cases (62 per cent) of this group were examples of active endocarditis on leaflets thickened by a previous rheumatic infection. In 46 cases no blood culture was made. In the remaining 62, 11 were negative, 28 showed *Streptococcus viridans*, 7 *Streptococcus hemolyticus*, 14 streptococci of undetermined type, and 2 cocci of undetermined type. The negative cultures were chiefly from cases in which only one blood culture was made.

Secondary anemia of some degree was found in almost every instance in which the blood was examined. The average weight of 101 spleens was 419 gm. The weight was 400 gm. or more in 43.5 per cent, and 600 gm. or more in 19.4 per cent of the cases. Infarction of the spleen or kidneys occurred in 69.4 per cent, only a little above the incidence of infarction in the acute group (Table IV).

In 19 of the 108 cases studied (17.6 per cent) all the glomeruli were either entirely normal, or they showed only minor alterations, such as a slight increase in the prominence of the endothelium or a little uneven thickening of the capillary basement membrane. They were

classified as normal unless the alterations were definite, as shown in Figure 1. The group with normal glomeruli show no distinctive difference in the duration of the disease, but there is evidence that cardiac decompensation was a more important factor than septicemia in causing death. The average weight of the spleen in these 19 cases was 291 gm., as compared with 419 gm. for the entire group, and 449 gm. for the group in which glomerular lesions were present. In a general way, the size of the spleen depends upon the duration and the intensity of the infective process, and it may be inferred that glomerular lesions depend upon these same factors in the disease.

Diffuse glomerulitis was found in 64.8 per cent, with embolic lesions in 35.2 per cent, and alone in 29.6 per cent. Embolic lesions were found in 52.8 per cent, with diffuse glomerulitis in 35.2 per cent, and alone in 17.6 per cent. In the group with embolic lesions only, the average weight of 17 spleens was 331 gm., as compared with an average of 480 gm. in those with diffuse glomerulitis. There were 3 very large spleens in the purely embolic group, but there is a suggestion that the septicemia was less intense than in those with diffuse glomerulitis.

It is to be noted that both embolic and diffuse glomerular lesions were present in 35.2 per cent. Very frequently a glomerulus showed an embolic lesion in one lobule and diffuse glomerulitis in the others. The lesions did not exclude one another except when the embolic lesion was very large, and when the glomerulitis was as pronounced as in clinical glomerulonephritis.

B. Secondary Bacterial Endocarditis

Sixty-nine examples of this group were studied (Table III). Endocarditis was always a distinctly secondary process and was never recognized clinically. It was not considered the cause of death in any instance, and was presumably a terminal infection. It was associated with definite infectious processes in 42 instances, of which the most frequent were acute endometritis (14), and lobar pneumonia (9). In these the duration given in the table is that of the major illness and therefore it represents the maximum possible duration of the endocardial disease. In 27 instances endocarditis appeared as a terminal complication of some non-infective process,

TABLE II

Subacute Bacterial Endocarditis *

Number	Age	Sex	Duration	Old valvular defect	Bood culture	Weight of spleen	Glomerular lesions						Comment
							Embolic			Diffuse	Cres-cents	Normal	
							Infarcts	Hyaline	Fibrous				
	years					*gm.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
11–76.....	25	M	3 mo.	−	o	?	−	o	o	100	25	o	Chronic glomerulonephritis
12–131....	33	M	3 mo.	+	−	420	−	o	o	100	10	o	Acute glomerulonephritis
13–165....	33	M	3 wk. +	+	−	595	−	o	o	100	o	o	
13–190....	35	F	?	+	s.	296	−	o	o	100	o	o	
14–49.....	25	M	3 wk. +	+	o	very large	+	o	o	100	o	o	Acute glomerulonephritis
15–97.....	29	F	9 wk.	−	o	large	+	o	o	100	o	o	Acute glomerulonephritis
16–89.....	30	M	?	+	s.	594	−	4	7	4	85	o	Acute extracapillary glomerulonephritis
16–124....	45	M	5 mo.	−	s.v.	325	+	5	5	100	o	o	
16–203....	61	M	?	+	o	220	−	2	8	o	o	90	
16–413....	38	M	3 mo.	+	o	600	+	2	10	o	3	85	
17–36.....	29	F	6 mo.	+	s.v.	260	+	4	20	o	o	76	
17–174....	28	M	?	+	−	340	−	o	o	o	o	100	
17–202....	25	M	1½ yr.	+	o	1400	+	o	o	100	o	o	Chronic glomerulonephritis
17–260....	25	M	7 mo.	+	s.h.	650	+	25	50	25	6	o	
18–102....	23	M	3 mo.	−	o	440	+	o	o	100	o	o	
18–122....	30	M	3½ mo.	−	o	680	+	o	o	100	o	o	Acute glomerulonephritis
18–175....	34	M	8 mo.	−	o	500	−	9	52	84	9	o	
17–28.....	29	M	1 yr.	+	s.h.	360	+	o	o	100	o	o	
19–141....	55	M	6 wk.	−	−	325	+	5	o	100	o	o	
19–161....	23	F	8 mo.	−	s.v.	285	+	10	86	4	o	o	Uremia
19–264....	29	M	?	−	o	365	−	o	o	100	10	o	Chronic glomerulonephritis
19–276....	16	M	?	+	s.h.	200	+	o	o	100	o	o	

20–88.....	51	M	6 wk.	–	o	314	+	o	o	100	o	o	
20–165....	43	M	6 mo.	+	o	325	+	o	2	100	o	o	
20–296....	16	M	7 wk.	–	–	700	+	o	o	100	o	o	
20–344....	12	F	5½ mo.	–	o	190	+	10	66	o	o	24	
20–368....	18	M	6½ mo.	–	s.v.	310	+	34	17	35	24	o	
21–45.....	55	M	4 mo.	–	s.v.	310	+	27	8	8	10	48	
21–283....	24	M	7 mo.	–	s.v.	400	+	2	35	63	5	o	
21–414....	50	M	2 mo.	+	o	large	+	o	2	o	o	98	
21–468....	36	M	6 mo.	–	–	800	+	2	51	o	20	26	
21–513....	42	M	10 wk.	+	s.v.	170	–	o	o	o	o	100	
22–554....	43	M	5 mo.	–	o	310	–	2	67	o	12	19	Uremia
23–390....	45	M	?	+	o	480	–	90	o	100	10	o	Uremia
23–508....	76	M	7 wk.	+	o	360	–	2	22	o	o	76	
24–4.....	30	M	5 mo.	–	s.v.	?	+	37	o	95	3	o	
24–91.....	39	M	3 mo. ?	+	s.	670	+	6	7	90	o	10	
24–121....	59	M	8 mo. ?	+	coc.	400	–	o	o	o	o	100	
24–241....	23	F	3 mo.	+	s.v.	240	+	5	28	o	o	67	
24–605....	35	M	3 mo. +	+	–	730	+	6	34	50	20	20	
24–769....	14	F	7 mo.	+	s.v.	135	+	8	44	o	30	20	
24–770....	10	F	5 mo. +	+	s.v.	325	+	11	21	70	o	o	
25–142....	4	F	6 wk.	–	s.	80	+	6	o	o	o	94	
25–362....	42	M	1 mo. +	–	s.v.	280	–	o	o	10	o	90	
25–589....	30	F	2 mo. +	–	o	700	+	5	o	87	7	o	
25–789....	45	M	6 wk. +	+	–	135	+	5	o	o	o	95	
25–565....	56	M	11 mo.	–	o	170	–	o	o	o	o	100	
25–630....	30	M	3 mo.	+	s.v.	700	–	o	o	100	o	o	
25–888....	35	F	6 wk.	–	s.h.	445	+	2	o	100	o	o	
25–1031...	52	M	2 mo.	+	s.	160	+	o	o	o	o	100	
26–191....	17	F	8 mo.	+	o	300	+	6	17	97	1	o	
26–201....	46	M	3 mo.	+	s.v.	625	+	13	14	73	1	o	

* Under blood culture "o" indicates that no culture was made; s.v. *Streptococcus viridans;* s.h. *Streptococcus hemolyticus*: s. streptococcus of undetermined type; coc. coccus of undetermined type.

TABLE II (continued)

Number	Age	Sex	Duration	Old valvular defect	Blood culture	Weight of spleen	Glomerular lesions						Comment
							Embolic			Diffuse	Cres-cents	Normal	
							Infarcts	Hyaline	Fibrous				
	years					*gm.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
26–639....	17	M	3 mo. +	+	coc.	400	+	0	0	100	0	0	
26–654....	34	M	6 mo. +	+	−	1040	+	0	0	100	0	0	
26–760....	55	M	?	+	0	650	−	5	61	0	0	34	
26–859....	38	F	4 mo. ?	+	0	150	−	0	0	0	0	100	
26–1082...	43	M	6 mo.	+	0	875	+	0	0	100	0	0	
26–1136...	46	M	3 mo.	−	s.v.	500	−	0	0	100	0	0	Acute glomerulonephritis
27–59.....	12	F	2 mo.	−	0	175	−	0	0	0	0	100	
27–535....	26	M	9 mo.	+	s.	180	+	12	77	0	4	7	Uremia
27–749....	34	M	1 yr.	+	0	540	+	0	0	0	0	100	
27–768....	44	M	8½ mo.	+	0	820	+	0	0	100	0	0	
27–795....	25	F	9 mo.	+	0	400	+	0	0	0	0	100	
27–861....	29	M	10 wk.	−	s.v.	840	+	0	2	100	0	0	
27–1280...	34	M	7 wk.	+	s.	375	+	0	0	100	0	0	
28–1.....	48	F	6 wk.	+	s.h.	200	+	40	0	99	3	1	
28–74.....	31	F	2 mo.	+	s.	620	+	0	0	100	0	0	
28–91.....	35	M	?	+	0	600	+	1	5	100	0	0	
28–315....	39	M	7 mo.	+	0	400	+	0	0	0	0	100	
28–320....	29	M	3 mo.	+	0	100	+	0	0	0	0	100	
28–1098...	30	F	5 mo.	−	s.v.	450	−	5	0	100	0	0	
28–1234...	42	M	2 mo.	+	s.h.	320	−	0	0	100	0	0	
28–1243...	46	M	7 mo.	−	−	400	+	3	3	0	0	94	
29–137....	60	M	6 mo.	−	s.	450	+	0	0	100	0	0	
29–449....	35	F	1 yr.	−	0	350	+	18	29	65	5	0	
29–746....	63	M	5 mo.	−	−	425	+	0	0	100	0	0	
29–1085...	26	F	3 mo.	−	s.v.	250	+	26	7	0	5	61	

29–1146...	38	M	?	+	o	175	–	o	o	100	o	o	
29–1251...	30	M	3 mo.	+	o	large	–	o	o	100	o	o	
29–1503...	27	F	5 mo.	–	o	600	+	52	8	50	33	o	Uremia
30–352....	19	F	3 mo.	+	s.h.	447	+	o	o	100	o	o	
30–574....	37	F	3 mo.	–	o	270	–	10	o	o	o	90	
30–609....	29	M	3 mo.	+	s.	380	+	o	o	o	o	100	
30–819....	27	F	9 wk.	–	o	850	–	62	o	17	1	20	
30–909....	41	F	13 wk.	+	s.v.	390	+	o	o	100	o	o	Acute glomerulonephritis
30–912....	40	F	?	–	o	614	+	o	o	o	o	100	
30–1205...	45	M	7 mo.	–	o	475	+	o	o	o	o	100	
30–1232...	31	F	3 mo.	+	o	300	+	o	o	o	o	100	
30–1377...	15	M	7 mo.	+	s.	110	–	o	o	o	o	100	
32–325....	35	M	9 mo.	+	o	340	–	10	6	100	20	o	
30–1522...	24	M	9½ mo.	–	s.	375	+	4	66	30	o	o	
30–1641...	22	M	4 mo.	+	o	640	+	8	16	100	6	o	
30–1900...	23	M	4 mo.	+	o	530	+	18	15	92	25	o	
30–1946...	21	F	10 wk.	+	s.v.	310	+	o	o	o	o	100	
31–33.....	60	M	?	+	o	100	–	o	o	o	o	100	
31–45.....	66	F	9 wk.	+	o	637	+	50	14	36	40	o	
31–115....	38	F	14 wk.	–	s.v.	320	+	4	13	90	2	o	
31–158....	18	F	3 mo.	–	s.v.	422	+	24	1	o	o	75	
31–646....	17	M	7 mo.	+	s.	220	+	o	o	o	o	100	
31–838....	40	F	1 yr.	+	s.v.	350	+	16	o	100	7	o	
31–1210...	18	F	2 mo.	+	s.v.	300	+	3	4	100	1	o	
31–242....	29	F	3 mo. +	+	s.v.	210	+	o	o	100	o	o	
31–455....	61	F	8 mo.	+	s.	225	+	28	55	50	2	o	
31–1370...	19	F	14 mo.	+	o	250	+	65	34	90	2	o	
31–1209...	44	M	4 mo.	–	o	325	–	5	o	100	o	o	
31–1259...	51	M	6 mo.	+	s.v.	202	–	3	2	o	o	95	
32–138....	28	F	?	+	s.v.	475	–	o	o	5	o	95	
31–954....	35	M	?	+	s.v.	445	+	8	13	85	25	o	

TABLE III

Secondary Acute Bacterial Endocarditis *

Number	Age	Sex	Duration	Old valvular defect	Blood culture	Weight of spleen	Glomerular lesions			Cause of death
							Infarcts	Embolic	Diffuse	
						gm.				
13–180	63	M	2 mo.	+	o	small	–	–	–	Carcinoma of stomach
14–255	39	M	4 wk.	+	o	285	–	–	++	Lobar pneumonia
16–89	30	M	2 wk.	+	s.	594	–	++	–	Erysipelas
16–358	62	M	?	–	o	75	–	–	–	Peritonitis, postoperative
17–233	38	F	?	–	o	200	–	–	–	Abdominal tumor
18–39	39	M	?	–	–	160	+	–	–	Pernicious anemia
18–53	47	M	?	+	o	130	–	–	–	Carcinoma of stomach
19–94	46	F	9 days	–	s.h.	125	+	–	–	Peritonitis, postoperative
20–122	63	M	?	–	–	235	+	–	+	Postoperative shock
20–209	56	M	?	–	s.h.	300	+	–	–	Carcinoma of stomach, peritonitis
20–267	25	F	?	–	o	?	+	–	+	Acute endometritis
20–388	38	F	?	–	o	810	+	–	–	Leukemia
20–428	65	F	1 wk.	–	o	230	+	–	–	Pneumonia
21–150	21	F	4 wk.	–	–	120	–	–	–	Acute endometritis
21–165	65	M	1 mo.	–	o	65	–	–	–	Carcinoma of stomach
21–237	36	F	3 wk.	–	s.h.	310	+	–	+	Acute endometritis
21–307	54	M	10 days	–	o	220	–	–	–	Primary hypertension
21–559	28	M	14 wk.	+	s.v.	140	–	–	+	Ulcerative colitis
22–134	46	F	9 days	–	s.h.	500	–	–	–	Influenzal pneumonia
22–149	68	M	?	–	o	80	–	–	–	Carcinoma of prostate
22–229	23	F	?	–	o	140	–	–	–	Peritonitis
24–566	26	M	?	–	pn.	425	+	–	–	Thrombosis of cavernous sinus
24–628	5½	M	?	–	o	?	–	–	–	Appendicitis
24–807	20	F	7 mo. ?	+	s.h.	225	+	–	+++	Lupus erythematosus, acute disseminated
25–103	68	M	3 wk.	–	o	205	+	–	–	Multiple fractures
25–1019	28	M	2 wk.	–	o	225	–	–	–	Lobar pneumonia
26–565	20	F	6 wk.	–	s.h.	170	–	–	–	Acute endometritis
26–1134	43	M	?	–	o	1180	–	–	+	Leukemia, tricuspid valve
26–1154	38	M	?	–	o	140	–	–	+	Inguinal granuloma
27–149	21	F	8 days	–	o	160	–	–	–	Peritonitis
27–177	57	M	4 days	–	o	250	+	–	–	Hypertension
27–392	27	F	?	–	s.v.	250	–	–	–	Sinus thrombosis

27–404	27	F	?	–	o	55	–	–	–	Actinomycosis
27–530	15	M	18 days	–	st.	570	+	–	+	Acute osteomyelitis, pyemia
27–565	70	M	?	–	o	?	–	–	–	Abdominal abscess
27–608	36	F	2 mo.	–	o	475	+	–	+	Acute endometritis
27–761	35	F	?	+	o	275	–	–	+	Carcinoma of uterus
27–1266	36	F	3 mo.	–	o	355	–	–	+	Abscess of lung
28–37	28	F	2 wk.	+	o	950	+	–	–	Acute endometritis
28–66	57	M	?	–	o	185	+	–	+	Carcinoma of stomach
28–284	40	M	2 days	–	o	550	–	–	–	Lobar pneumonia
28–695	55	M	5 wk.	–	o	300	+	–	–	Lobar pneumonia
28–921	55	F	?	–	o	150	+	–	–	Carcinoma of stomach
28–1103	48	F	?	–	o	190	–	–	–	Carcinoma of cervix
29–55	77	F	2 wk.	–	o	130	–	–	–	Erysipelas
29–887	69	M	?	+	o	?	+	–	–	Carcinoma of stomach
29–1796	54	F	10 days	–	o	200	+	–	–	Postoperative infection
30–77	52	F	?	–	o	542	–	–	–	Leukemia
30–198	1½	F	2 wk.	–	o	39	–	–	–	Peritonitis
30–506	55	F	?	–	o	112	–	–	+	Carcinoma of ovary
30–546	38	F	7 wk.	–	s.h.	820	–	–	+	Acute endometritis
30–879	7	F	10 days	–	o	70	–	–	–	Osteomyelitis
30–1100	37	F	2 mo.	–	st.	350	+	–	–	Empyema
30–1315	52	M	3 wk.	–	o	500	+	–	–	Appendicitis
30–1353	41	F	?	–	o	242	–	–	+	Tubo-ovarian abscess
30–1679	19	F	3 wk.	–	o	444	+	–	–	Acute endometritis
30–1792	39	M	?	–	o	145	–	–	+	Lobar pneumonia
31–10	34	F	1 wk.	–	o	160	–	+	–	Acute endometritis
31–72	26	F	2 wk.	–	o	610	+	–	+	Acute endometritis
31–87	40	M	5 days	–	o	98	–	–	–	Gangrene, *B. Welchii* infection
31–170	44	M	?	–	o	840	–	–	–	Lobar pneumonia
31–215	25	F	2 wk.	–	o	465	+	–	++	Acute endometritis
31–793	22	M	3 wk.	–	s.h.	700	+	–	–	Fracture of skull
31–877	50	M	?	–	o	110	–	–	–	Lobar pneumonia
31–887	58	M	?	+	pn.	560	–	–	+	Lobar pneumonia
31–903	66	M	?	–	o	350	+	–	+	Carcinoma of stomach
31–912	29	F	1 mo.	–	o	370	+	+	+	Acute endometritis
31–1003	22	F	1 mo.	–	s.h.	447	+	–	–	Acute endometritis
31–2020	23	F	18 days	–	s.h.	480	+	+	+	Acute endometritis

* Under blood culture "o" indicates that no blood culture was made; s.v. *Streptococcus viridans;* s.h. *Streptococcus hemolyticus;* s. streptococcus of undetermined type; st. staphylococcus; and pn. pneumococcus.

usually of chronic nature. The duration of the endocarditis in this group could seldom be determined. There were 8 cases of carcinoma of the stomach.

In 59 instances there had been no previous valvular disease, but in 10 the vegetations formed on old healed rheumatic valves. Blood cultures, which were made in only 20 cases, showed *Streptococcus hemolyticus* 10, *Streptococcus viridans* 2, streptococcus 1, pneumococcus 2, staphylococcus 2, negative 3.

The average weight of 59 adult spleens, not including leukemia, was 307 gm. Seventeen of these weighed over 400 gm. The largest spleens were found in association with acute endometritis, the average weight being 448 gm. The rôle of infection in causing enlargement of the spleen is obvious. The average weight of the adult spleen in 21 instances where endocarditis was a terminal complication of a chronic disease was 207 gm., exclusive of leukemia. In 38 cases where death was due entirely to an acute infectious process, the average weight of the spleen was 363 gm.

Acute diffuse glomerulitis was found in 23 instances (33.3 per cent), 17 times in association with definite infectious processes and 6 times with chronic diseases. The average weight of the spleen in the cases with glomerular lesions was 352 gm., and in those without glomerular lesions 267 gm., but there were several very large spleens in cases without glomerulitis.

Embolic glomerular lesions were found in 4 cases. Infarction of the spleen or kidneys was present in 30 cases (43.4 per cent) and absent in 39.

A summary of some of the data reviewed in the preceding paragraphs is given in Table IV.

Diffuse Glomerulitis

This lesion was evidently recognized by Löhlein in 1910, since he noted that the capillary loops of many glomeruli were thicker and broader, the nuclei increased above the normal, and the lumens of the capillaries apparently filled with protoplasm. The lesion consists chiefly in an increase in the size and number of the endothelial cells, but there is also a thickening of the capillary basement membrane in many instances. The glomeruli are not enlarged, but they have an avascular solid appearance. The lumens of the

capillaries are decreased in size by encroachment of the endothelial cells and the basement membrane (Fig. 1). Sometimes the thickened basement membrane is more responsible than the endothelium for this appearance. Occasionally a few polymorphonuclear leukocytes are found in the capillaries. The lesion is not identical with clinical acute glomerulonephritis, since the capillary obstruction is much less pronounced and no intracapillary fibers are found, but the difference is chiefly in the intensity of the reaction. In association with subacute endocarditis (Table II) there were 7 examples of typical acute glomerulonephritis, and there were 2 other cases in which the lesion was nearly as prominent as in clinical acute glomerulonephritis. It may be safely inferred that the moderate

TABLE IV

A Comparison of the Four Forms of Acute Endocarditis with Respect to Glomerular Lesions, Size of Spleen, and Frequency of Infarction of the Spleen and Kidneys

Endocarditis	Number examined	Embolic lesions	Diffuse glomerulitis	Normal	Weight of spleen	Infarcts
		per cent	*per cent*	*per cent*	*gm.*	*per cent*
Rheumatic	104	2.9	22.2	77.8	0	0
Acute primary bacterial	56	7.1	28.6	66	316	61.7
Subacute bacterial	108	52.8	64.8	17.6	419	69.4
Secondary acute	69	5.8	33.3	63.7	307	43.4

diffuse glomerulitis, found so frequently with endocarditis, is a true glomerulonephritis differing only in intensity from the clinical form. Symptoms do not develop unless capillary obstruction is fairly pronounced.

Usually diffuse glomerulitis involves nearly all of the glomeruli, but in a few instances only a small proportion of them are affected. When associated with embolic lesions the lobules not involved in the embolism commonly show the characteristic diffuse reaction.

The causative factor in diffuse glomerulitis is evidently infection. Glomerulitis occurs in all forms of endocarditis but is much more frequent in the subacute form. Presumably its greater frequency in this group is due to the more prolonged and more severe septicemia. It was pointed out in a preceding paragraph that in sub-

acute endocarditis the average weight of the spleen is much greater (449 gm.) in cases where glomerular lesions are present than in those with normal glomeruli (291 gm.). In both forms of acute bacterial endocarditis the spleens are smaller and the incidence of glomerulitis is less than in the subacute type. Both the duration and the intensity of the infection are factors in the development of glomerulitis. Glomerulitis is more frequent than embolic lesions in subacute endocarditis.

Clinical acute glomerulonephritis in association with endocarditis is interpreted as the result of the infection responsible for the endocarditis, but chronic glomerulonephritis is evidently due to an antecedent infection. A contracted kidney cannot develop in the comparatively short course of endocarditis.

The Embolic Lesions

The embolic lesions were described by Löhlein in 1906[3] and 1910.[1] Baehr[4-8] has made important contributions to this subject in a series of papers published from 1912 to 1923. In his first communication, Löhlein interpreted the lesions as capillary thromboses, but in his second paper he explained them as small infarcts resulting from the lodgement of minute infected emboli detached from the valves of the heart. Both Baehr, and Fahr,[9] agree with this latter interpretation.

Löhlein found typical embolic lesions in each of 8 cases of "chronic ulcerative endocarditis," but noted great variations in the number of glomeruli involved. In 3 of his cases *Streptococcus viridans* was obtained in blood culture.

Baehr[4, 5] believed that only subacute streptococcus endocarditis is associated with embolic lesions. He found typical lesions in 21 of 25 such cases in which the blood culture was positive for *Streptococcus viridans*, and in 6 of 7 cases with negative blood cultures. The percentage of involved glomeruli varied from 2 to 75 in the group. No lesions were found in 1 instance of influenzal and another of gonorrheal endocarditis. In 54 instances of acute endocarditis "due to ordinary pathogenic bacteria" no embolic lesions were found. Baehr seems to distinguish "acute" from "subacute" endocarditis by the kind of organism obtained in blood culture rather than by the duration of the disease. In 75 cases of verrucous

(rheumatic) endocarditis Baehr and Sacks[8] found no embolic lesions, but they mentioned 2 cases with thromboses of capillary loops.

Miller and Branch[10] described typical embolic lesions in a case of endocarditis of fifty days duration caused by a hemophilic bacillus resembling *B. influenzae.*

In our experience, embolic lesions are not limited to subacute *Streptococcus viridans* endocarditis. They may be caused by *Streptococcus hemolyticus*, and they are also found in acute endocarditis. In 3 of 104 cases of rheumatic endocarditis capillary thromboses were found which correspond to the early stages of the embolic lesions. In 4 of 56 cases of acute endocarditis (duration of less than six weeks), and in 4 of 69 cases of secondary endocarditis embolic lesions were found. Most of these were small capillary thromboses, but some were typical large embolic lesions. In our series of 108 cases of subacute bacterial endocarditis embolic lesions were found in only 52.8 per cent. This is a surprisingly low figure in comparison with Baehr's observations, but it is a much larger series and includes all cases which came to autopsy from 1911 to 1932. The percentage of involved glomeruli varied from 2 to 99 per cent. It was over 50 per cent in 18, and 5 per cent or less in 8 cases.

In 1923 Baehr mentioned 2 instances in which embolic glomerulonephritis occurred in the absence of endocarditis. Fahr[9] also recorded a similar case. In our series there was 1 such instance in a man 27 years of age, who underwent nephrectomy for a tuberculous kidney and died nine days later from infection of the wound, erysipelas and septicemia. The kidney removed at operation showed no glomerular disease, but the one obtained at postmortem showed acute glomerulonephritis and numerous typical embolic lesions. There was no endocarditis.

Fahr believes that embolic lesions occurring in the absence of endocarditis are caused by clumps of bacteria which lodge in the capillaries, but it is improbable that masses of bacteria in the circulating blood are of sufficient size and firmness to produce infarction. They no doubt lodge in the capillaries, but the necrosis of the glomerulus is probably a toxic effect rather than the result of infarction.

It may be concluded that embolic glomerulonephritis occurs chiefly in association with subacute streptococcic endocarditis, but it may also develop occasionally in rheumatic endocarditis, in acute

forms of endocarditis, in endocarditis due to organisms other than streptococci, and even in the absence of endocarditis of any kind.

What are the factors which influence the formation of embolic lesions in bacterial endocarditis? It is obvious that the duration of the infection is of great importance, since the lesions are rare in the acute cases of less than six weeks duration and frequent in those of longer standing (see Table IV); but beyond the six weeks period no definite influence of the time element can be made out. The lesions are just as frequent at two or three months as they are after six months. They are usually caused by *Streptococcus viridans*, but may be produced by *Streptococcus hemolyticus* and other organisms. Staphylococcic infections, however, cause glomerular abscesses and not embolic lesions of the type under discussion. There is no evident relation to gross infarction in the spleen and kidneys, since infarction is nearly as frequent in the acute as in the subacute group (Table IV). However, in the subacute group embolic lesions were found in 57 per cent of those with infarcts and in 35 per cent of those without infarcts. The vegetations on the valves are commonly larger and more numerous in subacute than in acute endocarditis, and we would therefore expect a smaller number of involved glomeruli in the latter, but not a complete absence of the lesions, since gross infarction occurs readily. The fact that a certain duration of the infection is necessary suggests that immune bodies may play a rôle in the formation of the glomerular lesions. We do not understand why cases of subacute bacterial endocarditis that are similar in clinical and anatomical features show variations from 0 to 99 per cent in the number of glomeruli with embolic lesions.

The Structure of the Glomerular Lesions

Two distinct types of lesions are found in embolic glomerulonephritis, the fresh or hyaline, and the fibrous. In sections stained with hematoxylin-eosin the hyaline lesions take a deep eosin stain and appear homogeneous, while the fibrous lesions stain less intensely with eosin and have a fibrous structure. In such preparations one gets the impression that the hyaline lesions are gradually transformed into the fibrous. Baehr came to this conclusion and interpreted the hyaline as recent and the fibrous as old lesions. The azocarmine stain, however, shows a sharp contrast between

the hyaline and the fibrous alterations — the former staining a bright red, the latter a deep blue. There are no transitions between the two forms, and their pathogenesis is quite distinct.

The Hyaline Lesions: In its simplest form, this is an intracapillary thrombus (Fig. 2). A homogeneous mass is found which distends a long segment of a capillary but does not cause necrosis of its walls. A somewhat larger lesion (Fig. 3) shows necrosis of portions of the capillary wall including the overlying epithelial cells. When the thrombus does not distend the capillary, both the endothelial cells and the basement membrane remain intact. The outlines of the thrombosed capillaries are readily seen in many large lesions. In Figures 4 and 5 the capillaries appear as solid cords with persistent epithelial nuclei between them. After the capillary walls have undergone necrosis the thrombosed capillaries become confluent and their outlines are no longer recognizable. Some of the coagulated material may be carried into the capsular space or even into the first part of the tubule. The thrombosis may occur in any part of the glomerulus, the hilum, central portion, or periphery; and one or more lobules may be involved. Rarely the entire glomerulus is thrombosed. Not infrequently thrombosed capillaries are scattered irregularly throughout the glomerulus.

In the great majority of the kidneys from subacute endocarditis small intracapillary thromboses were found in addition to the typical large lesions, and in a few instances only the small lesions were present. The peripheral parts of large lesions frequently pass over into distinct intracapillary thrombi. The typical large lesions are clearly of the same nature and origin as the small intracapillary thrombi. The capillary thrombi seen occasionally in acute glomerulonephritis are similar to small embolic lesions in structure and origin.

The hyaline material composing the thrombus stains a pale blue with Weigert's fibrin stain and occasionally a few fibers may be demonstrated with this stain. It is evidently formed from the constituents of the blood. When the capillary walls become necrotic they disintegrate and disappear and do not become a part of the hyaline mass.

The process is capillary thrombosis and not embolism. The glomerular lobules are not infarcted in the sense that the blood is cut off from distal vessels by occlusion of a proximal branch. True

infarction of a glomerulus is seen in hypertension with acute uremia, and is caused by necrosis and sudden occlusion of the afferent arteriole. The glomerulus shows dilated capillaries with necrosis of their walls but no thrombi. There is no resemblance to embolic glomerulonephritis.

Clawson [11] obtained true embolic glomerulonephritis in rabbits by intracardiac injections of finely ground agar seeded with streptococci. Inflammatory lesions of low intensity developed about the masses of bacteria but no infarction of the glomeruli resulted.

The lesions are embolic in the sense that they are caused by the lodgement of minute infected emboli in the glomerular capillaries. Toxic substances arising from the mass of bacteria are presumably responsible for capillary thrombosis and necrosis. The focal character of the lesion indicates that the bacterial bodies and not their soluble toxins are responsible. Neither Löhlein nor Fahr was able to demonstrate bacteria in the embolic lesions, but Baehr succeeded in demonstrating bacteria in 5 instances. Even after prolonged search we have been unable to find bacteria in our material, although all the cases showing large fresh lesions were investigated.

In the case of embolic lesions without endocarditis it is easily possible that bacteria taken up by polymorphonuclear leukocytes and subsequently liberated in the glomerular capillaries by necrosis of the leukocytes, may produce focal lesions. Fahr demonstrated bacteria in the lesions in his case of embolic glomerulonephritis without endocarditis.

What is the ultimate fate of the fresh hyaline lesion? Does it disintegrate and disappear, or is it converted into a fibrous lesion? Baehr is definitely committed to the latter view. He believes that fibroblasts from without invade the hyaline lesion and convert it into fibrous tissue. Fahr believes that the hyaline lesion becomes fibrous but does not indicate how the process comes about. In preparations stained with hematoxylin and eosin one gets the impression that there are transitions between the fresh hyaline and the fibrous lesions, but with the azocarmine stain it is clear that this is not the case. The hyaline lesions become necrotic, disintegrate and disappear. Around some of them there is a thickening of the basement membrane which produces a fibrous appearance, but this alteration is around the lesion and not within it.

In the subacute group there were 13 instances in which only the

fresh hyaline lesions were present. Five of these were of only six weeks duration, but in 2 the endocarditis had been present for five months, and in 1 for one year. The presence of fresh lesions only does not necessarily indicate that the disease is of short duration. In 3 instances only fibrous lesions were found. The duration of these cases was two months, ten weeks, and six months respectively. In the great majority of cases both types of lesions are present.

The Fibrous Lesions: The fibrous lesions are a little less frequent than the fresh hyaline. With azocarmine the former are colored a deep blue and the latter a bright red. The contrast between the two types is very striking. Evidently a somewhat longer time is required for the development of the fibrous lesions than for the fresh hyaline, since the former are rarely found in any form of acute endocarditis. They were numerous in one case of seven weeks duration, but usually they are not present in cases of less than three months standing. The lesions vary in size from small focal fibrotic areas to diffuse fibrosis of the entire glomerulus. In its earliest stages (Fig. 6) the fibrous lesion shows a thickening of the basement membrane of a group of capillaries. There is no increase of endothelial nuclei, no thrombosis, and no necrosis. The basement membranes increase in thickness at the expense of the capillary lumens. The fibers are all thickened basement membranes. There is no invasion of fibroblasts. When the membrane is moderately thickened it gives the staining reactions of collagenous fibers.

The large diffuse lesions (Figs. 7 and 8) are formed in the same way as the focal fibrous areas. Occasionally small, fresh, hyaline lesions are found in a diffuse fibrous glomerulus, but the fibrous lesions do not develop from the hyaline form. The thick fibers may be followed directly into normal basement membranes (Fig. 8). The end result of a fibrous lesion is a dense mass of thick membranes which occludes the capillaries completely. Endothelial and epithelial nuclei persist for a long time but finally disappear and a hyaline glomerulus results.

The focal character of the lesion suggests that it may be caused by the lodgement of bacteria in the capillaries, but bacteria have never been demonstrated. If bacteria are responsible we must believe that the injury is not so intense as in the case of the hyaline lesions. No capillary thrombi are formed and no necrosis occurs, but a type of proliferative inflammation is induced.

Complete closure of a glomerulus results in atrophy of its associated tubule. When a large proportion of the glomeruli are obliterated by embolic lesions, death results from uremia. In 5 cases of subacute bacterial endocarditis the embolic lesions were so extensive that uremia developed. In 3 of these, large fibrous lesions were responsible and in 1, large, fresh, hyaline lesions. In the fifth case the glomeruli were destroyed by large, fresh, hyaline lesions and large, fibrous crescents which compressed the glomeruli.

In the subacute group there were 7 instances of acute glomerulonephritis and 3 of chronic glomerulonephritis in which there was ample anatomical evidence of severe renal insufficiency. Therefore, in the subacute group there were 15 cases with anatomical evidence of marked renal insufficiency, 10 due to diffuse glomerulonephritis, and 5 due to embolic lesions. Schiele [12] reported 2 cases of embolic glomerulonephritis terminating in uremia. In 1 of these there was no endocarditis. In 77 cases of subacute streptococcus endocarditis Baehr [6] reported nine deaths from uremia, two of which were due to acute and seven to chronic glomerulonephritis.

Epithelial crescents are a prominent feature in subacute bacterial endocarditis. They were found in 31 per cent, always in association with diffuse or embolic lesions. In 3 instances they were associated with diffuse glomerulitis, in 2 with embolic lesions, and in 29 with both diffuse and embolic lesions. Shortly after its formation, fine fibers appear between the epithelial cells of the crescent (Fig. 9). These increase in size and number until the entire crescent has a dense fibrous structure (Fig. 10). The fibers are formed entirely under the influence of the epithelial cells; there is no invasion of the crescent by fibroblasts from without. After the fibers have become coarse they give the staining reactions of collagen. Fibrous crescents compress the glomeruli and stop the capillary circulation. The result of this compression is a collapse of the capillaries and a marked thickening of their basement membranes. Finally the thickened membranes fuse into a rather homogeneous mass. Many glomeruli are obliterated by epithelial crescents.

It is to be noted that in glomerulonephritis fibers form inside the capillaries from endothelial cells and in the crescents from mesenchymal epithelial cells. The term "fibroblast" can no longer be restricted to cells of the connective tissues.

Summary

Two forms of glomerulitis, namely, diffuse and embolic, are found in association with endocarditis.

Diffuse glomerulitis was found with the various types of endocarditis as follows: acute rheumatic 22.2 per cent, acute primary bacterial 28.6 per cent, subacute bacterial 64.8 per cent, and secondary acute 33.3 per cent. It is characterized by an increase in the number and size of the endothelial cells and often by thickening of the capillary basement membrane. The extent of capillary obstruction is usually much less than in clinical acute glomerulonephritis, but in 7 instances of subacute endocarditis glomerulitis had reached the clinical level. Diffuse glomerulitis bears some relation to the intensity and duration of septicemia.

Embolic, or focal glomerulitis was found in the different forms of endocarditis as follows: acute rheumatic 2.9 per cent, acute primary bacterial 7.1 per cent, subacute 52.8 per cent, and secondary acute 5.8 per cent. In 1 instance there was no endocarditis.

Two distinct types of embolic lesions occur — the fresh hyaline and the fibrous.

The fresh hyaline lesion in its simplest form is a capillary thrombosis and all the smaller lesions are readily recognized as such. The larger lesions are composed of many thrombosed capillaries which may be identified until the capillary walls have undergone necrosis. The hyaline lesion is not an infarct but a thrombosis and necrosis of capillaries resulting from the lodgement of bacteria. The necrotic portion of the glomerulus disintegrates and disappears.

The fibrous lesion is a reaction characterized by a marked growth of the basement membranes of the capillaries. The thickened membranes obliterate the capillaries and give the glomerulus a fibrous structure. The fibers are formed entirely from basement membranes; there is no invasion by fibroblasts from without. The fibrous lesion, like the fresh hyaline, may involve one or more lobules or the entire glomerulus. It develops independently of the fresh hyaline lesions.

In subacute bacterial endocarditis there were 15 instances of severe renal insufficiency, of which 5 were due to embolic glomerulonephritis, 7 to acute and 3 to chronic glomerulonephritis.

The fresh, hyaline, embolic lesions develop earlier than the fibrous

and may be found at any time during the course of the disease. The frequent absence of embolic lesions in typical clinical examples of subacute bacterial endocarditis has not been explained.

Diffuse glomerulitis is frequently found in association with embolic lesions.

Epithelial crescents frequently cause atrophy of the glomerular tufts by compression. Fibers form between the epithelial cells and convert the crescent into a dense fibrous structure. These fibers are of epithelial origin.

In the glomeruli, fibers which later give the staining reactions of collagen are formed from three distinct sources — intracapillary fibers from the endothelial cells, fibers formed from thickened capillary basement membranes, and fibers formed by the epithelial cells of the crescents.

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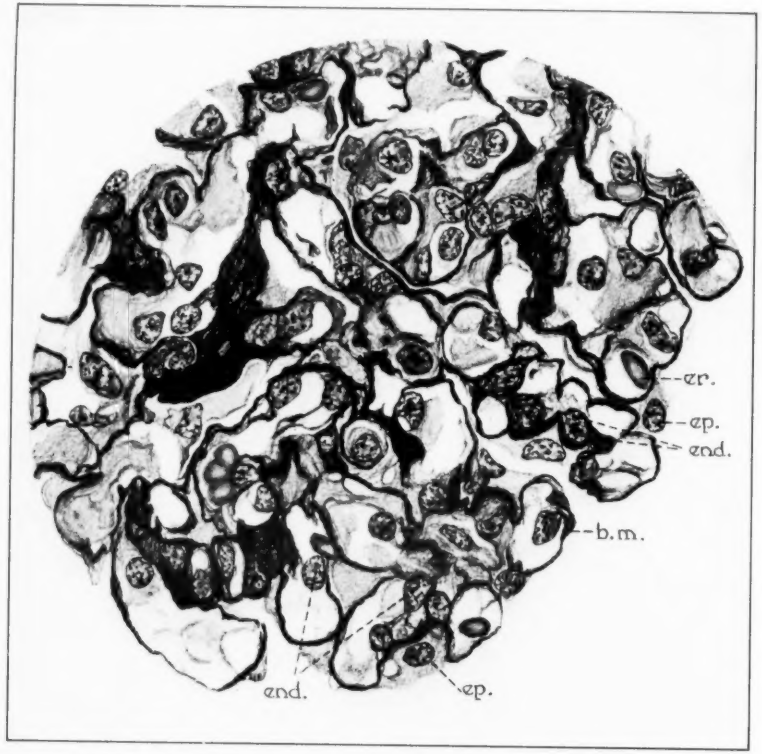
DESCRIPTION OF PLATES

PLATE 106

FIG. 1. 31–1209. Diffuse glomerulitis. Part of a glomerulus from a case of subacute bacterial endocarditis. Azocarmine stain. There is a definite increase in the number and size of the endothelial cells (end.) and there is also a moderate uneven thickening of the capillary basement membrane (b. m.). The lumens of the capillaries are definitely narrowed, but the obstruction is not so prominent as in clinical acute glomerulonephritis. × 750.

FIG. 2. 27–445. Small, fresh, hyaline, embolic lesion — capillary thrombosis. Small portion of a glomerulus from a case of acute rheumatic endocarditis. Azocarmine stain. The thrombus distends the capillaries but there is no necrosis of the capillary wall. The small embolic lesions in subacute bacterial endocarditis are all of this type. Ep., epithelial cell; end., endothelial cell; b. m., capillary basement membrane; t., thrombus. × 750.

FIG. 3. 30–819. A part of a large, fresh, hyaline, embolic lesion from a case of subacute bacterial endocarditis. Azocarmine stain. The thrombotic material (t.) fills some of the capillaries completely and others partially. The capillary basement membrane (b. m.) is present in some places but absent where necrosis has occurred. Endothelial nuclei (end.) may persist after the basement membrane and epithelial cells have disintegrated. The outlines of the capillaries may usually be recognized in large, fresh, hyaline lesions. Ep., epithelial cell. × 750.



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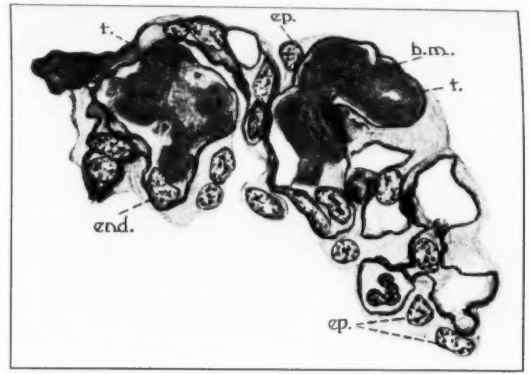

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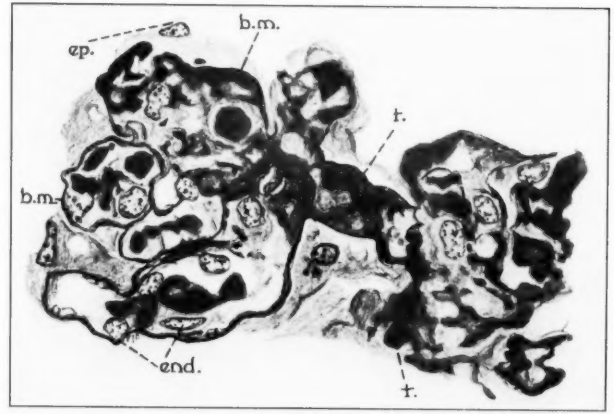

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Glomerular Lesions Associated with Endocarditis

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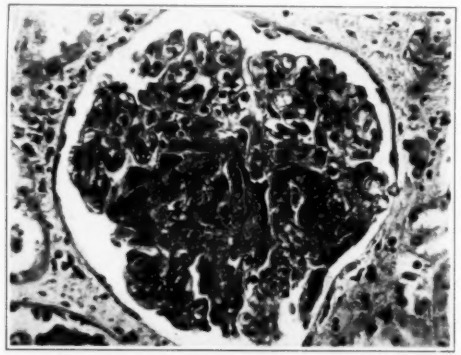
PLATE 107

FIG. 4. Fresh, hyaline, embolic lesion. From the same case as Fig. 3. Hematoxylin-eosin stain. The individual thrombosed capillaries may be seen. × 400.

FIG. 5. Higher magnification of a small part of the lesion shown in Fig. 4. Note the nuclei of epithelial cells between the thrombosed capillaries.

FIG. 6. 16–80. Subacute bacterial endocarditis. Area from a glomerulus showing a small fibrous lesion. Azocarmine stain. The hyaline bands (h.) are formed by thickening of the basement membranes of the capillaries. Normal basement membranes are continuous with thickened ones. The endothelial cells (end.) are increased in number and size in capillaries outside of the fibrotic area. B. m., normal capillary basement membrane; ep., epithelial cells. × 750.

FIG. 7. 30–1522. Diffuse fibrous lesion. From subacute bacterial endocarditis. Azocarmine stain. The glomerulus is largely replaced by coarse fibers. × 400.



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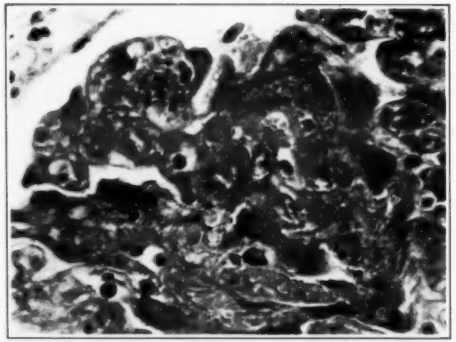

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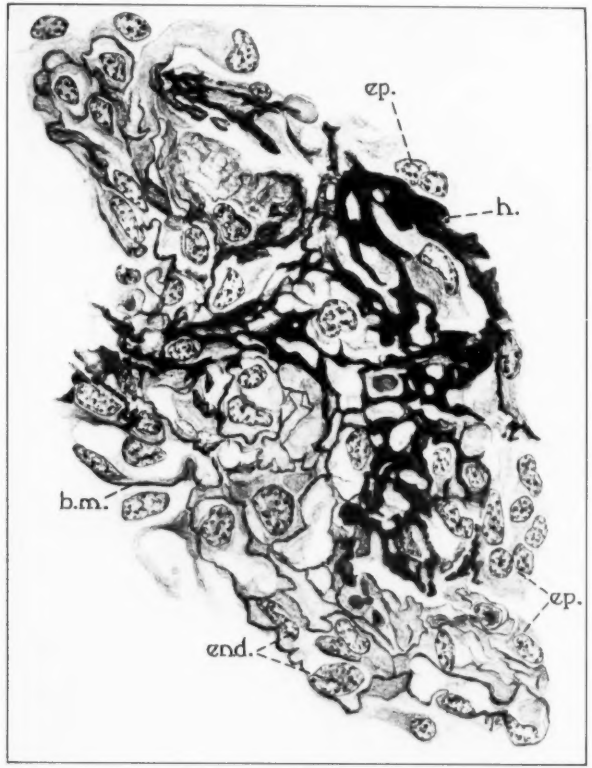

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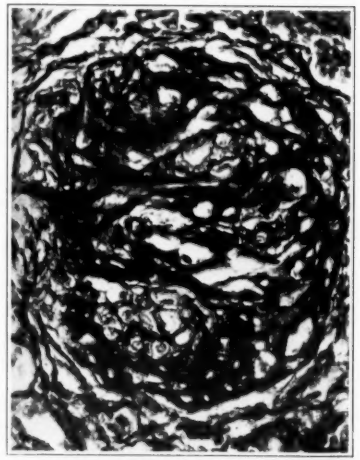

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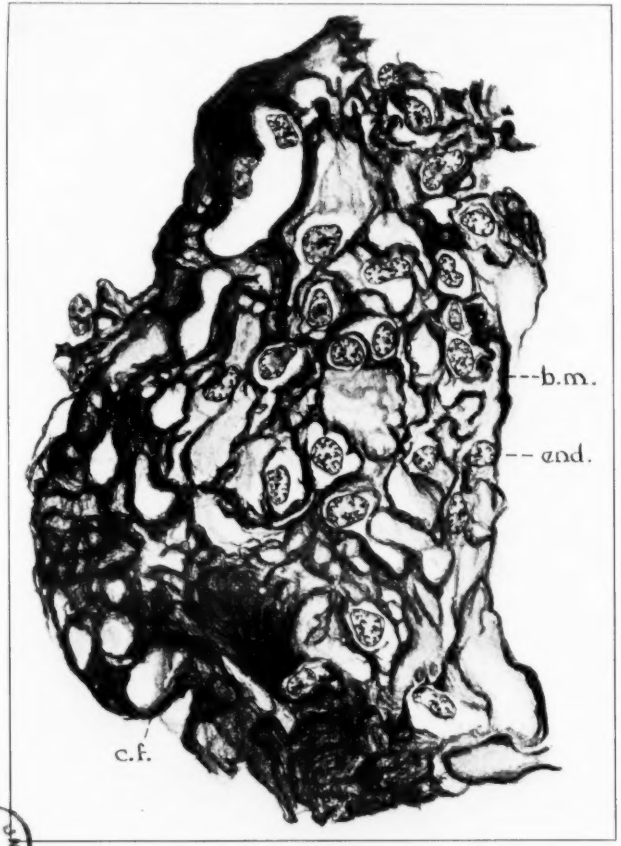
Glomerular Lesions Associated with Endocarditis

PLATE 108

FIG. 8. From the same case as Fig. 7. Azocarmine stain. The coarse fibers (c. f.) are continuous with definite capillary basement membranes. The majority of the nuclei are probably endothelial (end.). × 750.

FIG. 9. 16–89. Epithelial crescent from subacute bacterial endocarditis. Azocarmine stain. Small fibers (f.) are shown among the epithelial cells (ep.). C. b. m., capsular basement membrane. × 750.

FIG. 10. 31–455. Epithelial crescent from subacute bacterial endocarditis. Azocarmine stain. The epithelial cells (c. ep.) are largely replaced by coarse fibers (f.). C. b. m., capsular basement membrane. × 750.



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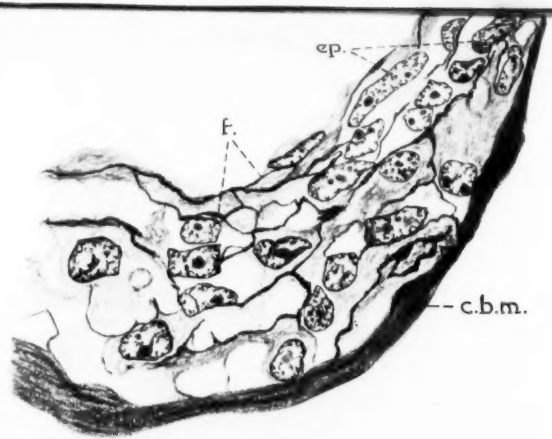

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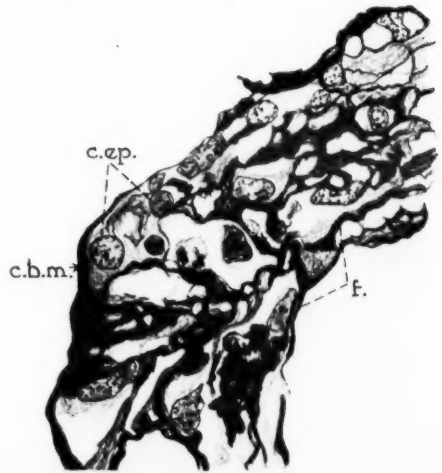

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Bell

Glomerular Lesions Associated with Endocarditis

GLOMERULAR CHANGES IN THE KIDNEYS OF RABBITS AND MONKEYS INDUCED BY URANIUM NITRATE, MERCURIC CHLORIDE AND POTASSIUM BICHROMATE *

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The injurious effects of the salts of uranium, mercury and chromium on the epithelium of the renal tubules have long been recognized. Of these substances uranium nitrate particularly has been employed as a nephrotoxin by numerous experimental workers in attempts to solve the difficult problems of renal function. All of the poisons mentioned cause definite and unmistakable lesions in the proximal convoluted tubules. Although certain cytological changes have long been noted in the glomeruli of kidneys injured by the salts of heavy metals, these alterations are histologically much less prominent than in the tubules, and possibly for this reason almost universally have been considered of less significance than the outspoken and widespread tubular necrosis, in spite of certain clinical evidence to the contrary.

The usual tissue stains show degeneration and even necrosis of both capillary and capsular glomerular epithelium, often a granular albuminous substance in the capsular space, occasional intra- and extraglomerular hemorrhages, diminution, absence or excess of blood within the glomerular capillaries during the acute stage of injury by uranium and similar substances. The histological state of the capillary basement membrane cannot well be studied by ordinary methods and has therefore generally been neglected. As the lesions of chemical nephrosis become chronic there is seen a proliferation of the capsular epithelium sometimes forming epithelial crescents, thickening of Bowman's membrane and apparent atrophy, fibrosis, or even hyalinization of the glomerular tufts.

A brief review of the literature on experimental nephritis will lend emphasis to the preceding statements.

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Takayasu [1] failed to see any very marked glomerular lesions in a study of sixty rabbits poisoned with uranium nitrate, potassium bichromate and mercuric chloride, in which changes termed tubular nephritis by Schlayer and Hedinger [2] had been found. Christian [3] described for the first time the occurrence of "hyaline droplets" within the glomerular capillary walls of rabbits receiving uranium nitrate and potassium bichromate. Christian felt the presence of such bodies must indicate that considerable injury to the capillaries of the glomeruli had been brought about by the chemicals employed. In later studies Christian, Smith and Walker,[4] and Christian and O'Hare [5] noted that the hyaline droplets were followed by more advanced degenerative changes consisting of hyaline thrombus formation, hemorrhage, bleeding in the glomeruli with dilatation of the capsular space, proliferation of capillary endothelium and also of the capsular epithelium. Baehr [6] states that Mackenzie was able to demonstrate occasional hemorrhages or "blood cysts" in the glomeruli of certain rabbits injected with uranium nitrate. Suzuki [7] also noted glomerular hemorrhage and necrosis in one uranium-damaged kidney. Scheel [8] mentions the appearance of necrosis of the glomeruli in uranium nephritis but gives no further details. After Christian the first notable attempt to ascertain the nature of glomerular lesions induced by uranium nitrate was made by Baehr. By subcutaneous administration he obtained, in one of ten rabbits, a collapse and thickening of the glomerular capillaries in which there was a homogeneous protoplasmic substance. The capsular space contained albumin, while the loops were blended with Bowman's capsule and formed epithelial crescents. More constant results were obtained after injection of very small quantities of uranium directly into the renal artery on one side. By this technique 40 to 50 per cent of glomeruli were affected. According to Baehr the evolution of the glomerular lesions could be divided into four stages. The first was characterized by coagulation necrosis, failure of the endothelial nuclei to stain, a diminution of blood and the presence of a reticulated coagulated substance in the capillaries. The epithelial covering of the loops was often seen to be necrotic. From the damaged vessels there was an extravasation of blood or plasma into the capsular space. In some instances entire glomeruli were affected but in others the injury was limited to certain loops. The afferent arterioles were plugged with granular masses similar

to those in the capillaries of the tufts. The second stage was marked by a swelling and proliferation of glomerular and capsular epithelium with the formation of syncytial structures and multinucleated giant cells, the two types of cells forming a network leading to obliteration of the capsular space. In the third phase the capillaries and vasa afferentia became more permeable, filled with blood, and sometimes formed large blood lacunae. The final stage was characterized by hyaline degeneration of the markedly injured loops with partial adhesion to the capsule. Baehr concluded that a glomerulonephritis could be produced artificially, in the manner described, with but slight evidence of tubular damage. The overgrowth of capsular epithelium was felt to be the result of stimulation by the exudate filtered through the glomeruli. Suzuki [9] in 1926 published the results of another series of experiments with uranium, in which he advances the opinion that the thickening of the glomerular loops has its origin in the formation of a connective tissue layer in the glomerular walls. Collapse of the glomeruli is the result of contraction of the connective tissue growth in the walls. He further states that there is sometimes proliferation and swelling of the glomerular endothelium. Suzuki makes the interesting observation that hyaline droplet degeneration occurs only in the epithelium, never in the ingrown connective tissue.

In the course of an experimental study concerned with acquired resistance of regenerated renal tubular epithelium to the nephrotoxic effects of uranium nitrate and sublimate, Hunter [10, 11] observed in addition to degeneration and necrosis of the glomerular and capsular epithelium of rabbits receiving uranium nitrate the quite frequent occurrence of a few erythrocytes in the subcapsular space. It was felt that these must have escaped from the capillaries largely by diapedesis, made possible by injury of the capillary endothelium. Albuminous material in the capsular space also indicated glomerular injury. Christian's observation concerning the constancy of hyaline droplets was confirmed. In the chronic stages of uranium nephritis there were invariably some alterations in the glomeruli, although the tubular lesions were always more prominent. After acute injury repair of the epithelium covering the tufts and Bowman's membrane began early and not infrequently was excessive, leading to the formation of epithelial crescents or even obliteration of the capsular space, glomerular atrophy and hyaline degenera-

tion. In other instances the capsular spaces appeared dilated and cystic with apparent atrophy of the glomeruli, but even in the most markedly scarred and contracted kidneys there were always some hypertrophied, blood-filled and apparently functioning glomeruli. With mercuric chloride glomerular damage, evidenced by necrosis of epithelium, intraglomerular hemorrhages and hyaline droplet formation, was less constant than in the uranium kidneys. In a comprehensive review of acute experimental nephritis MacNider [12] in summary states that there is more histological evidence of a glomerular injury from bichloride of mercury than from either uranium or the chromates, the injury consisting of intense congestion with or without an exudate, hyaline changes in the endothelium or thrombus formation. Regarding chromates he asserts that the participation of the glomeruli in the nephritis is late.

A distinct advance in the finer histopathology of the renal glomerulus came with the discovery by McGregor [13, 14] of the great value of Mallory's anilin blue-Heidenhain-azocarmine stain in differentiating the various glomerular elements, clearly outlining the capillary basement membrane, and thereby making it possible not only to study changes hitherto unperceived in this structure, but also to distinguish the endothelial and epithelial cells of the capillaries from each other. By means of this stain McGregor [15] later demonstrated characteristic basement membrane alterations in the kidney in essential hypertension. More recently Bell,[16, 17] employing the same staining method, has proved the existence of important alterations in the glomerular basement membrane of the kidney in lipoid nephrosis and in the toxemias of pregnancy. In each of the conditions named there is a tendency to pronounced thickening of the membrane.

Materials and Methods Employed in Present Investigation

As far as we have been able to find in the available literature, the azocarmine stain has never been used as an aid in determining the nature of glomerular alteration in experimental lesions induced by uranium, mercury and chromium salts. Therefore, after having first studied a considerable number of renal lesions in man and satisfying ourselves that for such purposes the azocarmine stain is a

valuable one, we next proceeded to investigate the histology of the normal rabbit and monkey kidney. Having thus established the normal it became necessary to obtain material for the investigation of possible pathological changes in kidneys injured by chemical nephrotoxins. The late Dr. A. S. Warthin generously made available the uranium and mercury rabbit material which was studied in his laboratory by the senior author. Sections of the uranium and bichloride series stained with hematoxylin-eosin, Van Gieson's stain and Mallory's phosphotungstic acid hematoxylin had been kept and were available for review. From the large number of kidneys seventy-two were selected and stained with azocarmine.

Histology of the Normal Rabbit Glomerulus

The structure is essentially the same as McGregor [13] has described for the human glomerulus, but exhibits certain minor differences which will have to be mentioned. The rabbit glomerulus is appreciably smaller than that of man, a factor which makes it more difficult to study. In most instances the afferent arteriole apparently passes only a short distance into the capsular space before breaking up into numerous capillaries. Commonly one sees most of the capillaries cut either in cross-section or tangentially (Fig. 1). The basement membrane is of uniform thickness except at points of branching of the capillaries where it widens out and becomes slightly irregular. In the normal state it stains deeply and evenly with the anilin blue component of the azocarmine stain. External to the membrane there is often visible a thin and slightly ragged granular substance, at times having a demonstrable connection with the cytoplasm of the capillary epithelium. On changing the focal plane of the oil immersion objective the membrane sometimes appears to consist of two layers, but often remains a single solid line. At the periphery of the glomerulus, where the loops are not so closely approximated as deeper within the tuft, the outline of individual loops can be followed with certainty. Using this method of examination it is found that the capillary walls exhibit comparatively little secondary undulation, thus differing from the human glomerulus in which secondary foldings are very common.

The glomerular epithelium, *i. e.*, the epithelium covering the capillaries, bears a strong resemblance to that in man. The nuclei

are quite large, of variable shape, depending upon their location, and poor in chromatin granules. The latter tend to assume a peripheral location within the nucleus and stain faintly with carmine. The cell cytoplasm is granular, often abundant, and stains a pale blue. Epithelial cells are rarely observed over the convexity of the peripheral loops and are most abundant in the interstices between the capillaries, where they are easily distinguished from connective tissue nuclei by size and staining characteristics and from the endothelial cells by their position external to the basement membrane. The average number per glomerulus, arrived at by counting all cells in five to ten glomeruli from the kidneys of four normal animals, was found to be 30.1 epithelial cells.

As in man the capillary endothelial cells are much less numerous, according to our calculations averaging 8.2 per glomerulus, giving an average ratio of 3.7 epithelial cells to each endothelial cell. The structure and staining reactions are quite comparable to the human kidney in that the nuclei are of variable shape (according to location), stain deeply with carmine, contain a larger number of chromatin granules than the epithelium and possess one or two prominent nucleoli. Cytoplasmic substance is rarely demonstrable. The nuclei of the endothelium lining the vasa afferentia and vasa efferentia are oval and distinctly elongated. Whenever glomeruli are cut so that the afferent or efferent vessels are visible for any appreciable distance, connective tissue cells with long, oval-shaped, heavily carmine-stained nuclei can be made out readily between the capillaries. More frequently such cells are lacking because the vessels soon break up into many branches between which connective tissue nuclei are rarely found.

The outer layer of Bowman's capsule is made up of bluish-staining fibrils which join with the stroma between the adjacent tubules, while the inner layer appears to be quite homogeneous and stains dark blue with azocarmine. In the normal rabbit the entire thickness of the membrane is not great. Covering its inner surface is a single layer of flattened epithelium with nuclei identical in appearance with those covering the capillaries. The epithelial lining is often not demonstrably a continuous one.*

* Wilbur [18] has recently reported the results of quantitative counts of the various glomerular cells in the human kidney. He finds that in the ordinary glomerulus from one-fourth to one-sixth of all nuclei, exclusive of those of leukocytes, are endothelial cells.

The Normal Monkey Glomerulus

Aside from its smaller size and consequently fewer number of all types of cells, the renal glomerulus of *Macacus rhesus* monkeys differs in no important respects from that of the human as described by McGregor.[13] A minor variation occurs in Bowman's membrane which in the monkey tends to be more wavy than in man. We have found the average number of glomerular endothelial cells per unit structure to be 10.2, with an average of 40 glomerular epithelial cells, giving a ratio of 3.9 epithelial cells to one endothelial cell. The average figure obtained for capsular epithelium was 7.9 (Table I).

Table I

Comparison of Glomerular Cells in Normal and Nephropathic Rabbit Kidneys

	Normal	Uranium nitrate		Mercuric chloride		Potassium bichromate
		Acute	Chronic	Acute	Chronic	Acute
Average number glomerular endothelial cells	8.2	5.7	8.2	3.6	5.8	6.9
Average number glomerular epithelial cells	30.1	23.4	28.1	16.1	30.7	27.3
Average ratio of endothelial to epithelial cells	3.7	4.1	3.6	4.4	5.2	3.9
Average number capsular epithelial cells	7.1	4.7	9.6	5.1	8.7	5.8

Comparison of Glomerular Cells in Normal and Nephropathic Monkey Kidneys

Normal		Acute potassium bichromate kidney	Chronic potassium bichromate kidney
Average number glomerular endothelial cells	10.2	8.0	14.6
Average number glomerular epithelial cells	40.0	34.2	57.7
Average ratio of endothelial to epithelial cells	3.9	4.3	3.9
Average number capsular epithelial cells	7.9	9.8	10.8

The Glomerular Changes Produced by Uranium Nitrate

The effect of this poison upon the glomeruli, as well as upon the convoluted tubules, varies somewhat according to the acuteness or chronicity of the nephropathy. Accordingly we have divided the kidneys into two groups, classing as acute those from eighteen rabbits which died or were killed between three and twenty-eight days, following one or at most two subcutaneous or intravenous injections of uranium nitrate (Table II). The chronic series includes seventeen animals receiving increasingly larger doses over a period of months and exhibiting definite chronic renal lesions, both grossly and microscopically.

In all of the kidneys of the acute group there are varying degrees of necrosis of the epithelium lining the proximal convoluted tubules, the extent and degree depending upon the size of the dose received. In the presence of visible tubular necrosis it is not unreasonable to expect alterations in the glomeruli as well, since the noxious agent must first have passed through them. It was hoped that the azocarmine stain would bring out something more than the well known effects upon the capillary endothelium and epithelium.

As shown in Table II all of the changes demonstrable with ordinary stains were recognized in azocarmine preparations as well. But in addition to these (obvious cellular necrosis, swelling and desquamation of epithelium, a general decrease of blood in the capillaries, frequent occurrence of a finely granular material in the capsular space, intraglomerular hemorrhages and an apparently normal Bowman's capsule), we have found other morphological disturbances apparently hitherto undescribed, particularly in the capillary basement membrane. A more detailed account of this lesion will be given later.

As one phase of the problem it appeared worth while to count the actual number of glomerular and capsular cells. Such a task is tedious and unless the capillaries contain blood enough to distend them partially one cannot always distinguish epithelium and endothelium, even though the nuclei stain differently. One must also depend upon the relation of individual cells to the basement membrane as a means of identification. In order to minimize the chances of error we began with a study of normal rabbit and monkey kidneys

and did not attempt counts on pathological glomeruli until reasonably certain of success. Experience showed that if the counts were made on glomeruli having patent or blood-filled loops not more than five to ten need be counted in each animal. When only a part of a glomerulus was included in the section it was found necessary to dismiss it from consideration, even if the loops were patent, because the small number of cells often gave false ratios. Using the average ratio between endothelial and epithelial cells as a basis it was found unnecessary to measure the diameters of individual glomeruli since the dimensions naturally vary with the blood content and patency of the loops, as well as the point where it happens to be cut in sectioning. The constancy of the figures shown in Table II attests the soundness of this basis of computation. In the majority of acute uranium kidneys there is a decrease of approximately one-third in the number of glomerular epithelial and endothelial cells, leaving the ratio between them practically unchanged (Tables I and II). The diminution of capsular cells is subject to greater variability, accounted for in part by the differences in the size of the glomeruli examined.

The hyaline droplets described by Christian as occurring in the capillary walls are present in fourteen of the eighteen kidneys of the acute series. In sections stained with azocarmine these bodies are of different sizes but never of great bulk, and have a reddish or orange color, depending upon the focal plane of the oil immersion objective. Often they are seen to lie in the capillary walls but in our experience, as well as that of Suzuki, appear constantly within the cytoplasm of glomerular epithelial cells (Fig. 2). At times the location seems to be intranuclear as well. We have also observed the droplets within the lumina of the capillaries but, like Christian, are unable to state whether or not such a position is due to artefact.

Azocarmine stains all connective tissues and collagenous substances a deep and dark blue, a property of great importance in the study of the glomerular basement membrane which apparently is collagenous in nature. In the acute group there are with one exception varying degrees of alteration in the basement membrane. Most frequently the homogeneous membrane substance is split up into fine fibrils and these in turn are oftentimes either fragmented or give the impression of beading with alternate light and dark staining. The picture may be best compared to the appearance of a frayed

TABLE II

Acute Effects of Uranium Nitrate on Renal Glomeruli of Rabbits

No.	Total dosage	Duration of experiment	How administered	Average No. glomerular endothelial cells	Average No. glomerular epithelial cells	Average ratio of endo- to epithelial cells	Average No. capsular epithelial cells	Changes in glomerular basement membrane	Bowman's membrane	Intraglomerular hemorrhage	Glomerular blood content	"Droplet degeneration"
	gm.	*days*										
BFI	0.0005	4	Intravenous	5.1	20.5	4.0	4.2	Frayed, stains poorly	Negative	Few	Slight	Few
R–85 A	0.001	4	Subcutaneous	5.8	23.3	4.0	5.8	Poorly stained, frayed?	"	Many	Slight	Moderate
R–5–I	0.001	3	Intravenous	6.3	26.5	4.2	6.6	Negative	"	None	Moderate	Numerous
R–6–I	0.001	3	Intravenous	5.5	22.5	4.1	4.1	Split into layers, fragmented	"	Many 8.4%	Moderate	Numerous
BKI	0.0015	17	Intravenous	8.2	34.6	4.3	4.2	Slightly beaded and fuzzy	"	None	Marked	Few
BOI	0.0015	17	Intravenous	8.6	28.4	3.4	5.0	Slight splitting, stains well	"	Few	Marked	Numerous
BRI	0.0015	18	Intravenous	6.2	22.6	3.7	3.6	Frayed, beaded, stains poorly	"	Many	Slight	None
R–1–IV	0.002	3	Subcutaneous	4.2	19.0	4.4	7.0	Frayed in glomeruli with hemorrhage	"	Many	Slight	Few
R–2–IV	0.002	3	Subcutaneous	5.9	29.5	5.1	6.7	Frayed, split when hemorrhage present	"	Many	Moderate	Numerous
R–10 A	0.003	16	Subcutaneous	6.8	26.3	3.4	6.6	Negative	"	None	Marked	None
R–7 A	0.003	17	Subcutaneous	4.2	19.4	4.8	4.7	Split, fragmented, beaded	"	None	Moderate	Few
R–16 A	0.009	28	Subcutaneous	8.0	30.8	3.9	5.0	Beading, splitting and rupture	"	Few	Marked	None
R–17 A	0.009	25	Subcutaneous	6.4	26.2	4.0	2.0	Beading, splitting and rupture	"	Many 4.9%	Marked	Few
R–76 A	0.009	24	Subcutaneous	4.8	19.8	4.1	1.8	Faintly stained, thinner than normal	"	Few	Slight	None
R–77 A	0.009	26	Subcutaneous	5.0	19.6	3.8	2.6	Stains lightly and unevenly	"	Few 1.1 %	Slight	Few
R–79 A	0.009	24	Subcutaneous	3.4	15.0	4.5	4.6	Frayed and stains poorly	"	Few 2.0 %	Slight	Few
R–1 0.009	0.009	3	Subcutaneous	3.6	18.0	5.1	5.2	Beaded, unevenly stained, frayed	"	None	Slight	Numerous
R–2 0.009	0.009	6	Subcutaneous	5.0	20.5	4.2	6.6	Beaded, unevenly stained, frayed	"	Few	Slight	Numerous

Chronic Effects of Uranium Nitrate on Renal Glomeruli of Rabbits

R–2 0.009	0.009	6	Subcutaneous	5.0	20.5	4.2	6.6	Beaded, unevenly stained, frayed	"	Few	Slight	Numerous

Chronic Effects of Uranium Nitrate on Renal Glomeruli of Rabbits

R–9 A	0.303	157	Subcutaneous	–	–	–	–	Thickened 1 to 4 times, wrinkled, frayed and split	Markedly thickened	None	Moderate	Slight
R–5 A	0.223	128	Subcutaneous	–	–	–	–	Thickened 1 to 5 times, wrinkled and distinctly beaded	Moderate thickening	None	Slight	None
BGI	0.176	227	Intravenous	11.2	45.0	4.0	14.4	Thickened, split, beaded	Moderate	None	Slight	Few
R–15 A	0.136	127	Subcutaneous	16.6	18.6	1.1	15.0	Markedly beaded, wrinkled, slight splitting	Moderate	None	Slight	Few
BAI	0.1275	149	Intravenous	8.5	35.8	4.2	12.2	Thickened 2 to 3 times, fragmented, beaded and frayed	Marked	None	Slight	None
BLI	0.1275	162	Intravenous	7.0	28.2	4.0	7.4	Markedly frayed, some beading and thickening	Moderate	Few	Slight	None
R–12 A	0.127	115	Subcutaneous	6.0	22.1	3.6	10.6	Frayed and split, sometimes thickened	Marked	Few	Marked	Few
R–11 A	0.127	114	Subcutaneous	4.2	27.8	6.6	5.8	Thickening, fraying, beading	Marked	None	Moderate	Many
R–6 A	0.127	120	Subcutaneous	4.8	19.8	4.1	7.0	Beaded, split and wrinkled	Slight	None	Moderate	Numerous
BQI	0.0875	148	Intravenous	11.2	35.0	3.1	11.7	Split into layers, at times thickened	Marked	None	Moderate	Moderate
BXI	0.041	109	Intravenous	10.4	32.6	3.1	6.0	Marked fraying, beading and irregular thickening	Slight	None	Moderate	None
BTI	0.041	108	Intravenous	8.6	30.6	3.5	7.6	Split and beaded	Moderate	None	Slight	Slight
BJI	0.0035	91	Intravenous	8.2	24.2	2.9	11.2	Split, thickened, beaded	Moderate	None	Moderate	Few
R–84 A	0.121	88	Subcutaneous	8.0	27.4	3.4	14.8	Slight splitting and beading	Slight	Few	Moderate	Slight
R–83 A	0.121	88	Subcutaneous	7.1	24.8	3.4	8.5	Slight splitting and beading	Slight	Few	Moderate	Moderate
R–14 A	0.119	63	Subcutaneous	6.3	32.0	5.0	2.6	Frayed and split	Moderate	None	Slight	None
R–8 A	0.015	61	Subcutaneous	6.0	18.2	3.0	9.6	Frayed and beaded	Markedly thickened	None	Moderate	Few

cotton string (Fig. 3). In a few cases splitting and beading are absent, the membrane simply failing to stain well and appearing fuzzy. In these kidneys the intertubular connective tissue and Bowman's membrane stain well, indicating that the abnormal staining of the capillary basement membrane is not due to faulty technique. With the picture of the normal glomerulus constantly in mind we endeavored to avoid terming as pathological the fine, bluish, granular substance often visible over the external surface of the membrane and the slight irregularities in thickness and contour at points of capillary branching seen in entirely normal glomeruli. Still another source of error to be avoided was that of mistaking closely approximated basement membranes belonging to different loops for splitting or fraying.

During the acute stage the basement membrane does not give the impression of being appreciably thicker than normal. A single dose of uranium does not affect all glomeruli and those situated in the inner half of the cortex are regularly injured more than the tufts near the capsule. If this point is not kept in mind studies of the acute uranium kidney are apt to be misleading. Azocarmine also brings out the details of the intraglomerular hemorrhages so frequently seen in acute uranium poisoning. Often these occupy all except one or two loops and under low power magnification look like blood cysts. Examination under oil immersion reveals constant fragmentation and splitting of the basement membrane at the periphery of the hemorrhage, although one cannot always be certain that only one membrane is included (Fig. 3). Within the hemorrhage remnants of basement membrane enmeshed in fibrin and red blood cells are not infrequently seen. Since the hemorrhages occur almost as frequently following subcutaneous injections as after intravenous administration one can hardly escape the conviction that bleeding into the glomerular substance is an expression of injury to the capillary walls.

Repetition and increase of dosage of uranium nitrate will in time produce a striking pathological picture in the rabbit kidney. As previously described by Hunter [10] practically all of the original epithelium lining the proximal convoluted tubules is in time destroyed and replaced by flattened atypical cells which are resistant to the nephrotoxin. There is a stimulation of the interstitial connective tissue and finally a contracted, granular-surfaced kidney is

found at autopsy. In the chronic stage there are obvious glomerular alterations, such as apparent atrophy, decrease in blood content of the capillaries, dilatation of the capsular space with or without granular material in it, rarely intra- and extraglomerular hemorrhage, hyperplasia of the capsular epithelium with epithelial crescent formation, obvious thickening of Bowman's membrane with epithelial cell inclusions and hyaline droplet degeneration.

The kidneys of all of the seventeen rabbits in the chronic uranium group display more convincingly the same changes in the glomerular basement membrane as in the acute stage, with the additional finding of definite thickening and not infrequently wrinkling of the membrane. The latter is not surprising in view of the shrinkage of glomeruli (Fig. 5). In the two rabbits showing the greatest thickening (R-5 A and BAI) reddish, oval-shaped nuclei are buried within the heavy membrane. Their position, external to the wavy condensed portion of the basement membrane, points to an epithelial or connective tissue origin. It is significant that in each of the two rabbits mentioned the glomerular capillaries are distended with blood in spite of the great thickening of their walls (Fig.5). Hyaline droplets occur less frequently and in lesser numbers than in the acute phase, and again appear to lie both in the substance of the epithelial cells and in the basement membrane. Cell counts show that both endothelial and epithelial cells possess sufficient regenerative capacity to come back to approximately normal after having been reduced by about one-third during the acute stages of the disease. We are unable to discover any evidences of hyperplasia of the connective tissue cells accompanying the arterioles, and are of the opinion that such cells have nothing to do with collapse and atrophy of the glomeruli. Our observations lead us to believe that the small size of apparently atrophic tufts is due largely to collapse of the capillaries. Practically all nuclei remaining in such glomeruli have the appearance and staining characteristics of epithelium. The ability of glomerular epithelium and endothelium to regenerate in the presence of repeated damage by uranium suggests that these cells, as well as those lining the tubules, become somewhat tolerant to the drug. In addition to glomerular epithelial and endothelial cells both acute and chronic uranium kidneys frequently display intracapillary nuclei more heavily impregnated with carmine than endothelial cells and devoid of chromatin granules or nucleoli.

Hematoxylin-eosin and Van Gieson's stains reveal these bodies to be quite like the lymphocytes of the blood in larger vessels. Their presence in the glomerular capillaries is apparently of no pathological significance. In the absence of definite knowledge of the nature of these apparent nuclei it was considered inadvisable to include them in the counts of glomerular cells.

The Nephropathy Caused by Mercuric Chloride

In a previous paper Hunter [11] pointed out that the local corrosive action and irregularity of absorption after subcutaneous administration, the danger of thrombosis following intravenous injection and the excretion of mercury by the intestine, resulting in severe enterocolitis in itself often sufficient to kill the animal, makes sublimate an undesirable substance for the study of experimental renal lesions. It was found that the amount of mercuric chloride absorbed varied greatly, so that in certain cases several injections might be given without any very evident damage to the renal epithelium, while in other animals a like quantity provoked well marked chronic glomerulotubular lesions very similar to those produced by uranium. Glomerular injury, as evidenced by necrosis of epithelium, hemorrhages and hyaline droplet degeneration, was less frequent than in the kidneys of the uranium series.

The kidneys from many of the same animals used in the first experiments were employed in the present investigation. Of the fifteen selected for restudy four were classed as acute and eleven as chronic on the basis of the number of injections, the duration of the experiment and the pathological state of the kidney observed microscopically.

In each of the four acute cases the glomerular basement membrane exhibits degenerative changes identical with those already described for the acute uranium kidney, namely, splitting, fragmentation, beading and rupture. These phenomena are especially pronounced in R-2 0.015 (Fig. 6). In addition to extreme fraying of the glomerular basement membrane there is in this kidney marked diminution of both endothelial and epithelial cells, leaving the loops practically stripped of cells, the necrotic remains of which fill the capsular space. It is interesting to note that in spite of the very evident injury the capillaries are distended with unclotted blood. The three

remaining kidneys of this group likewise display a significant decrease in glomerular cells, together with varying degrees of injury to the basement membrane. In contrast to the high incidence of intraglomerular hemorrhages and droplet degeneration of the capillary epithelium in acute uranium kidneys, hemorrhage occurs in but one instance of acute mercuric nephropathy, while droplets are found in two of the four animals.

In the ten rabbits receiving repeated doses of mercury there is some degree of splitting, beading or alteration in the staining quality of the basement membrane in all but one. Thickening is much less frequent than in uranium nephropathy. In the chronic mercuric chloride kidney the endothelial cells increase from an average of 3.6 in the acute stage to 5.8 per glomerulus. The glomerular epithelium, after falling to half the normal number in the acute phase, regenerates to such an extent that at the end the normal is re-established and in several instances is actually increased (Table III). As for other pathological findings there is often but not always a slight to moderate thickening of Bowman's capsule, fairly constant hyaline droplet degeneration, decrease in capillary blood content, rarely hemorrhages in the glomerular substance and the same heavily stained lymphocytic nuclei within the capillaries present in uranium nephritis.

Experiments with Potassium Bichromate

Six rabbits and six monkeys (*Macacus rhesus*) were employed in these experiments (Table IV). Of the six rabbits four had but one dose of poison and died within one to eight days. The two remaining animals first received 1 cc. of a 2 per cent solution followed by three doses of 1.5 cc. each over a period of thirty-eight days. In none of the six did the bichromate cause more than acute degeneration and necrosis of the tubular epithelium. Study of the glomeruli in azocarmine preparations reveals changes quite like those in the acute uranium and bichloride lesions, with the exception of R-6 which died on the first day of the experiment. Intraglomerular bleeding and droplet formation occurs in only two instances.

In the monkey the results are quite similar except for M-5 and M-6 (Table IV), in whom definite chronic tubular lesions were produced. In both animals the glomerular endothelial and epithelial cells show a significant increase over the normal, at the

TABLE III

Acute Effects of Mercuric Chloride on Renal Glomeruli of Rabbits

No.	Total dosage	Duration of experiment	How administered	Average No. glomerular endothelial cells	Average No. glomerular epithelial cells	Average ratio of endothelial to epithelial cells	Average No. capsular epithelial cells	Changes in glomerular basement membrane	Bowman's membrane	Intraglomerular hemorrhage	Glomerular blood content	"Droplet degeneration"
	gm.	*days*										
R–2 0.015	0.026	3	Subcutaneous	1.8	6.2	3.7	4.5	Extreme fragmentation, splitting and rupture	Negative	Few	Marked	None
R–1 0.020	0.030	3	Subcutaneous	3.2	17.8	5.5	3.1	Fragmented, beaded, split	"	None	Slight	None
R–1 0.003 S	0.0036	8	Subcutaneous	4.6	22.5	4.9	7.2	Splitting and fragmentation	"	None	Moderate	Few
R–1 0.004 S	0.042	64	Subcutaneous	4.8	18.2	3.7	5.7	Fuzzy and beaded	"	None	Moderate	Few

Chronic Effects of Mercuric Chloride on Renal Glomeruli of Rabbits

R–1 0.006 S	0.0225	29	Subcutaneous Intravenous	8.1	36.1	4.6	6.2	Fuzzy and beaded	Slightly thickened	Few	Slight	Moderate
R–2 0.008 S	0.0298	32	Subcutaneous Intravenous	4.6	19.8	4.3	5.2	Thickened and beaded	"	None	Slight	None
R–2 0.002 I	0.012	32	Intravenous	6.0	32.8	5.5	6.8	Splitting	Moderately thickened	None	Slight	Numerous
R–2 0.005 S	0.018	35	Subcutaneous Intravenous	7.2	35.0	4.8	6.4	Fuzzy, split and beaded	"	None	Slight	Moderate
R–4 Int. I	0.021	43	Intravenous	4.6	23.8	5.2	8.8	Fuzzy and indefinite, beaded and fragmented	Slightly thickened	None	Slight	None
R–2 0.009	0.0498	44	Intravenous	6.8	40.6	5.9	16.2	No apparent changes	"	None	Slight	Numerous
R–1 0.007 S	0.0258	44	Subcutaneous Intravenous	6.0	31.8	5.5	4.0	Fuzzy	"	Few	Moderate	Moderate
R–2 Sub. II	0.0755	66	Subcutaneous	6.4	26.6	4.2	9.8	Frayed	Negative	None	Moderate	None
R–1 0.001 I	0.0270	82	Intravenous	6.6	42.8	6.5	17.6	Slight splitting and beading	Markedly thickened	None	Slight	Few
R–2 Int. I	0.0428	84	Intravenous	4.5	25.8	5.9	9.3	Wrinkling, slight beading	Slightly thickened	None	Slight	None
R–1 Sub. I	0.1128	87	Intravenous	4.0	22.6	5.7	6.0	Uneven, split, fragmented	Negative	None	Moderate	None

TABLE IV

Effects of Potassium Bichromate on Renal Glomeruli of Monkeys

		67	Intravenous	4.8	22.6	5.7	6.0	Uneven, split, fragmented	Negative	None	Moderate	None

TABLE IV

Effects of Potassium Bichromate on Renal Glomeruli of Monkeys

No.	Total dosage	Duration of experiment	How administered	Average No. glomerular endothelial cells	Average No. glomerular epithelial cells	Average ratio of endothelial to epithelial cells	Average No. capsular epithelial cells	Changes in glomerular basement membrane	Bowman's membrane	Intraglomerular hemorrhage	Glomerular blood content	"Droplet degeneration"
	gm.	*days*										
M–1	0.726	1	Subcutaneous	7.0	26.2	3.8	6.2	Stains poorly	Negative	None	Moderate	Numerous
M–2	0.200	1	Subcutaneous	6.8	30.2	4.6	6.0	Stains poorly, frayed, split, slightly beaded	"	None	Marked	Numerous
M–3	0.280	71	Subcutaneous	7.4	34.8	4.6	16.4	No apparent change	Slightly thickened	None	Moderate	Very few
M–4	0.060	22	Subcutaneous	10.8	45.8	4.3	10.6	Frayed and slightly beaded	"	None	Moderate	None
M–5	0.320	160	Subcutaneous	16.8	63.8	3.8	13.0	Frayed and beaded, wrinkled, thickened 2 to 3 times	"	None	Slight	Numerous
M–6	0.320	163	Subcutaneous	12.4	51.6	4.1	8.6	Thickening, beading, fraying	"	None	Slight	Numerous

Effects of Potassium Bichromate on Renal Glomeruli of Rabbits

R–6	0.040	1	Subcutaneous	9.5	33.5	3.5	6.0	No changes in basement membrane	Negative	None	Slight	Few
R–4	0.040	3	Subcutaneous	7.2	24.0	3.3	5.7	Slight splitting	"	None	Moderate	Numerous
R–2	0.040	5	Subcutaneous	6.0	29.0	5.1	6.7	Stains poorly, splitting and fragmentation	"	None	Moderate	Few
R–9	0.040	8	Subcutaneous	9.6	39.0	4.1	8.5	Split and beaded	"	Few	Moderate	None
R–19	0.110	38	Subcutaneous	4.6	16.4	3.5	3.6	Slight fraying, fragmentation	"	None	Slight	None
R–18	0.110	38	Subcutaneous	5.0	22.0	4.4	4.4	Slight beading and splitting	"	None	Moderate	None

same time preserving approximately the normal ratio (Table I). Although definitely damaged, the degenerative splitting, fragmentation and beading is less marked than in either the uranium or mercury nephropathies of rabbits.

A detailed description of the tubular involvement will be published as a separate paper.[19] It is sufficient to say here that bichromate affects the epithelium of both proximal and distal convoluted tubules and that the regenerated cells are resistant to the poison.

Discussion

From the standpoint of histopathology there is no doubt as to the most prominent seat of renal damage caused by the chemical substances employed in the preceding experiments. Unquestionably the tubular epithelium, particularly that of the proximal convoluted units, suffers severely and after having become necrotic and detached presents a striking picture of devastation. It is not surprising, therefore, that in the presence of clearly demonstrable tubular necrosis the comparatively slight and less easily proved glomerular changes have been regarded as relatively unimportant in accounting for the altered renal function brought about by certain chemical nephrotoxins. Perhaps this is in part a heritage handed down by the earlier experimentalists, who grouped nephrotoxic substances into two classes: (*a*) including cantharidin, arsenic and diphtheria toxin, was thought to produce deleterious effects by injury of the vascular apparatus of the kidney; while (*b*) other chemicals like uranium, sublimate and chromates, were regarded as tubular poisons.

In the foregoing pages we have attempted to gather from the literature the more important observations of glomerular injury evoked by uranium, bichloride and bichromate. There is general agreement that the changes in the glomeruli are purely degenerative during the acute stages of the process and that later both degenerative and proliferative phenomena may occur. Beyond this point there have been comparatively few attempts to analyze closely the state of the glomeruli after damaging the kidney with poisonous chemicals.

Recent years have brought significant advances in the physiology of renal function, and the modern theory of glomerular filtra-

tion-tubular reabsorption with certain modifications is now generally accepted. More recently still the notable contributions of McGregor [13, 14, 15] and Bell [16, 17] have aided materially in explaining the nature and significance of glomerular lesions in human renal diseases. It now appears that the state of the glomeruli is of much greater importance than the condition of the tubules in the pathological physiology of the kidney, and that changes in the latter are largely secondary to and dependent upon glomerular disease.

The purpose of the present study was to determine with the aid of newer staining methods all of the abnormalities in glomeruli made nephropathic by metallic salts. Besides confirming the findings of many other writers recorded in the literature, we have discovered another which we believe to be quite significant, namely, the nearly constant occurrence of demonstrable alterations in the glomerular basement membrane, manifested by a splitting, fraying, fragmentation and abnormal staining reaction in azocarmine preparations. Since the glomerulus is the first structure in the kidney with which circulating poisons come into contact, it is not surprising that some of its elements may be damaged during the filtration process by which urine is formed. Such injuries may be marked, as in glomerulonephritis, or of less apparent significance, such as the thickening of the basement membrane seen in essential hypertension, lipoid nephrosis and the toxemias of pregnancy. Glomerular injury may likewise occur when the substance brought to the kidney is a simple chemical poison. The lack of histological evidence of lesions is not proof that the glomeruli escape unscathed. Nor does the extensive tubular necrosis explain all of the clinical symptoms, as we shall point out presently.

Our investigation is limited to morphological glomerular alterations and we are not in a position to record from personal observations more than a few already well known facts concerning the effects of the poisons used in our experiments upon the volume output and constituents of urine. We have observed that while a transitory polyuria may follow a lethal dose of uranium or mercuric chloride, oliguria or even anuria soon ensue and may persist until death of the animal. The urine excreted is concentrated and for some days contains quantities of albumin, casts, and frequently small numbers of red blood cells. If the renal lesion becomes chronic the abnormal

materials in the urine are reduced or disappear. Certain of the chronic animals develop a polyuria.

For information regarding the blood chemistry in chemical nephropathies we turn to the literature. MacNider [20] has demonstrated repeatedly the occurrence of decreased phenolsulphonephthalein excretion, retention of blood urea, non-protein nitrogen, creatinin and lowered alkali reserve in dogs suffering from acute or chronic uranium nephritis. In certain of the chronic cases he noted structural changes of a chronic degenerative nature in the glomeruli, — fibrosis, obliteration of some capillary loops by connective tissue — sometimes leading to hyalinization, while other glomeruli showed partial obliteration of the loops with thickened walls and blood within the lumina.

The striking similarity of the disturbances in the blood and urine in acute and chronic clinical glomerulonephritis and sublimate nephrosis in the human, and acute and chronic uranium and bichloride nephropathy of experimental animals, has not been properly appreciated. The urinary manifestations have been ascribed mainly to tubular involvement. In view of our results we find it impossible to subscribe to this view. It is not unreasonable to expect abnormal urinary function in the presence of a structural alteration of the glomerular basement membrane and covering epithelium. In this connection it is interesting to note that while the regenerated tubular epithelium becomes resistant to the particular substance employed, there are at all stages degenerative phenomena in the glomeruli.

The concept of injury to the glomerular basement membrane, with resultant altered permeability as the factor chiefly responsible for the disturbances in renal function following administration of metallic salts, is in keeping with the modern theory of renal function. If the physiologists are correct, destruction of tubular epithelium should be followed by excretion of a large amount of urine of very low specific gravity. As everyone knows quite the contrary is found. Not only is there oliguria and concentration of the urine but a retention of nitrogenous products in the blood as well. Except for the absence of edema and uremia the picture is quite similar to acute and subacute human glomerulonephritis. This concept is supported by the work of Oliver and Shevky,[21] who noted in one of their frog experiments a repression of urine associated with a good flow of blood through the glomerular capillaries, interpreted as possible

evidence of increased density of the basement membrane. Richards[22] has observed an active glomerular circulation in the absence of urine formation in the kidney of a living frog poisoned with mercuric chloride. He explains the anuria as due to increased absorption of water by injured and consequently more permeable epithelium. Oliver and Shevky's experiments with the same animal show the tubular damage to be a complete and structural one and that, in the absence of complicating vascular spasm, there is a failure of absorption rather than an increased absorption. Moore and Hellman,[23] using the intravital method of Hayman and Starr [24] for determining the number of open glomeruli, have shown that acute mercurial nephrosis in the rabbit is not associated with a decrease of glomerular circulation. They agree with Richards that the anuria is due to an inability of damaged tubular epithelium to prevent a resorption of tubular urine. Bieter [25] has demonstrated experimentally the essential rôle of glomerular injury in albuminuria. Proteinuria could be produced readily in glomerular fish (*Ameriurus nebulosus* and *Anguilla rostrata*) by asphyxia or mercuric chloride. Aglomerular fish (*Opsanus tau*) treated in the same way showed a lack of albumin, indicating that the renal glomerulus is essential for albuminuria. Very recently Jensen and Apfelbach [26] have reported the production of pure renal insufficiency in dogs by injection of charcoal particles into the renal arteries with resultant glomerular infarction, nitrogen retention in the blood, decrease in the ability of the kidney to concentrate and dilute urine and a lowering of its specific gravity.

We believe the histological changes in the glomerular basement membrane brought out by the special stain employed in our experiments may supply the missing link in the chain of evidence pointing to primary glomerular injury as the cause of the disturbances in renal function and blood chemistry of chemical nephropathies. Cellular damage occurs in acutely affected kidneys but is later repaired, while the splitting and fragmentation of the basement membrane is present during all stages of the disease.

The renal lesions of uranium and other metallic salts are commonly termed nephritis, a designation not properly applicable to such forms of kidney damage. The term nephritis should be restricted to those forms of renal disease exhibiting evidence of either proliferative or exudative inflammation. It is quite true that in certain chronic chemical nephropathies the interstitial connective tissue is

increased, but this is purely a secondary feature. Our results show that the glomerular epithelium and endothelium regenerate after the initial injury, but the total is usually not greater than in normal controls. Proliferation of capsular epithelium occurs regularly and may just as properly be regarded as evidence of proliferative inflammation as it is in human glomerulonephritis. If by nephrosis one refers to that group of renal diseases predominantly degenerative in character, this designation would more nearly fit pathologically the lesions provoked by heavy metals than any other. Clinically, however, the blood and urine manifestations do not fit those of typical nephrosis and we are left without any suitable name for chemically produced renal diseases other than chemical nephropathies.

Summary

1. By a special staining method (azocarmine) distinct changes, interpreted as evidences of injury, can be demonstrated in the glomerular basement membrane of kidneys damaged by uranium nitrate, mercuric chloride and potassium bichromate.

2. The lesions in the basement membrane are purely degenerative in character, present in both acute and chronic stages of chemical nephropathies, and appear to be permanent. The renal glomerulus is more vulnerable to poisons than the tubules and fails to develop the same degree of increased resistance toward them.

3. The opinion is advanced that alterations in the basement membrane of the type described may play an important rôle in the renal disturbances induced by certain metallic salts.

4. Fibrosis and connective tissue hyperplasia are not responsible for the appearance of the glomeruli in chronic uranium and sublimate nephropathies.

5. The existence of other long recognized glomerular changes demonstrable with ordinary stains is affirmed.

6. Uranium nitrate and mercuric chloride produce more histological alterations in the glomeruli than potassium bichromate.

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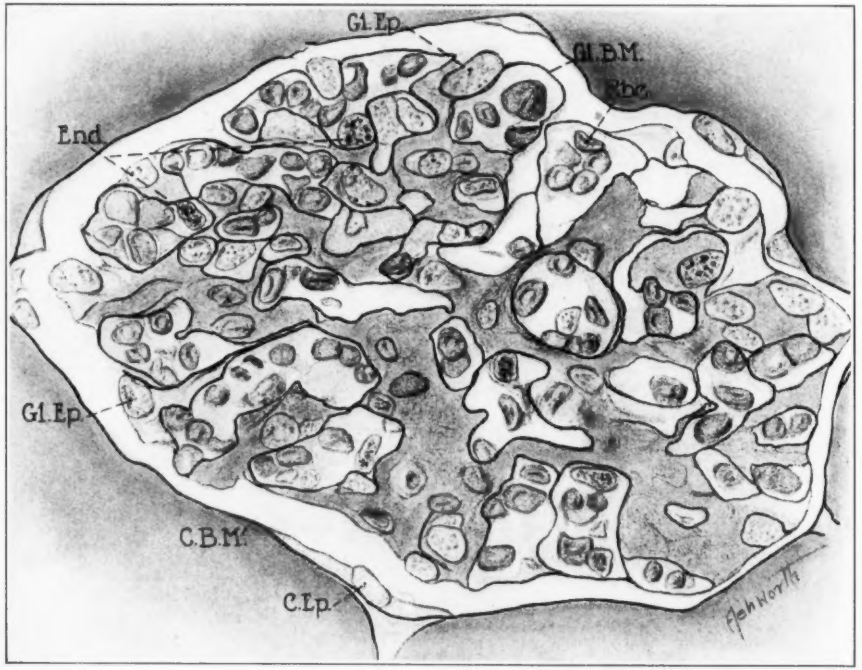
DESCRIPTION OF PLATES

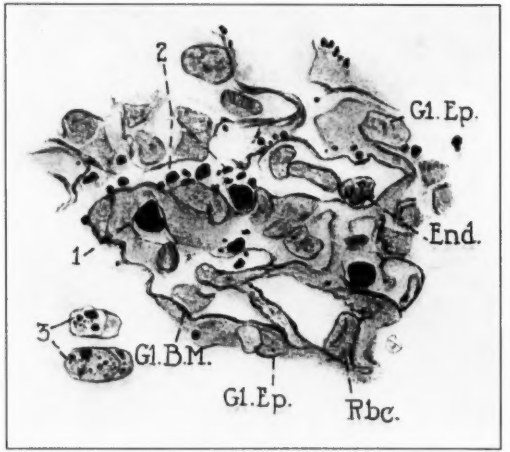
PLATE 109

FIG. 1. Camera lucida drawing of normal rabbit glomerulus stained with azocarmine (oil immersion). Gl. Ep. = glomerular epithelium; End. = endothelium; Gl. B.M. = glomerular basement membrane; Rbc. = red blood cells; C.Ep. = capsular epithelium; C.B.M. = capsular basement membrane. The glomerular basement membrane is of uniform thickness, evenly stained and shows comparatively little undulation. Epithelial cytoplasm is abundant.

FIG. 2. Several loops of glomerulus from R–BQI — given 0.0875 gm. uranium nitrate intravenously over period of 148 days. Camera lucida drawing, oil immersion. 1. = lymphocytes; 2 and 3 = so-called "hyaline droplets" in cytoplasm and nuclei of capillary epithelium; Gl. B.M. = slightly split glomerular basement membrane; End. = endothelium; Rbc. = red blood cells.



1



2

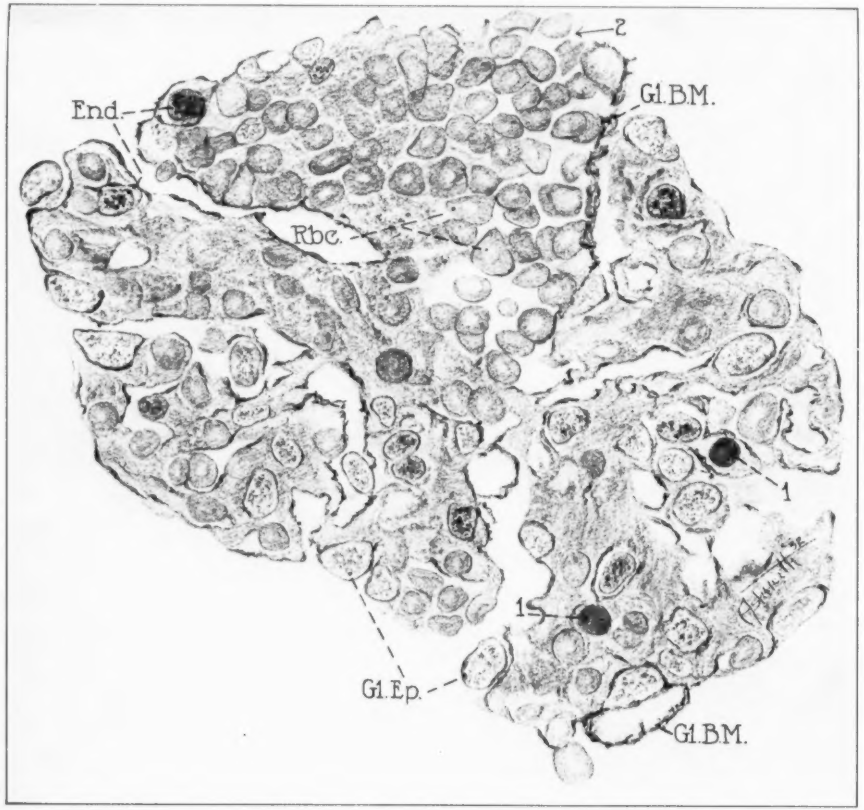
Hunter and Roberts

Glomerular Changes in Kidneys

PLATE 110

FIG. 3. Drawing of entire glomerulus of a rabbit receiving intravenous injection of 0.0005 gm. uranium nitrate and dying four days later. A small intraglomerular hemorrhage involving one loop is shown at the top with rupture of the frayed and fragmented basement membrane (Gl. B.M.) near point 2. Rbc. = erythrocytes; End. = capillary endothelium; Gl. Ep. = glomerular epithelium; 1 = lymphocytes in capillaries. (Oil immersion.)

FIG. 4. One loop of a glomerulus from rabbit with chronic uranium nephropathy displaying fraying and irregular thickening of capillary basement membrane (Gl. B.M.), and one epithelial cell (Gl. Ep.).



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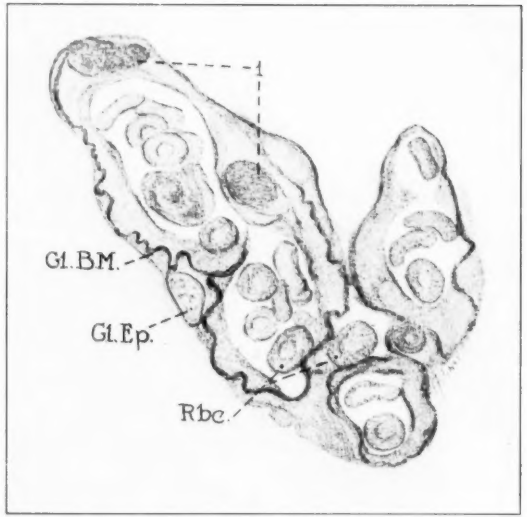

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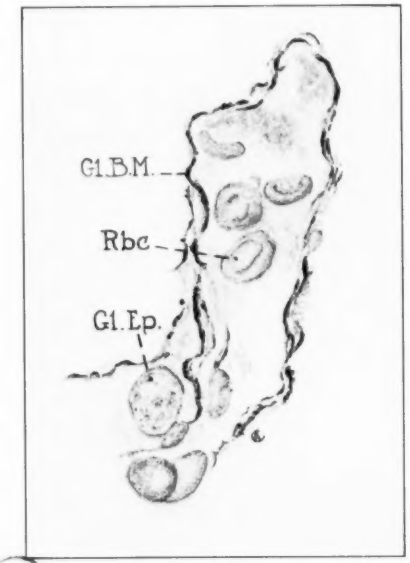
PLATE 111

FIG. 5. Portions of three loops of glomerulus from R–51A (see Table II). The glomerular basement membrane (Gl. B.M.) is markedly wrinkled, and both internal and external to it is a homogeneous non-granular substance taking the same stain as the original membrane but less deeply. 1 = nuclear inclusions within the thickened capillary wall; Gl. Ep. = glomerular epithelium; Rbc. = numerous erythrocytes in thick-walled but patent capillaries.

FIG. 6. Appearance of glomerulus in rabbit dying three days after subcutaneous injection of 0.026 gm. $HgCl_2$. Oil immersion, camera lucida drawing. Gl. B.M. = extreme fraying and fragmentation of glomerular basement membrane. Gl. Ep. and End. = glomerular epithelial and endothelial cells both greatly reduced in number. 1 = necrotic cellular débris filling capsular space, apparently derived from glomerular epithelium; the capillaries are distended with erythrocytes, Rbc.; C. Ep. = capsular epithelium; C.B.M. = capsular basement membrane.



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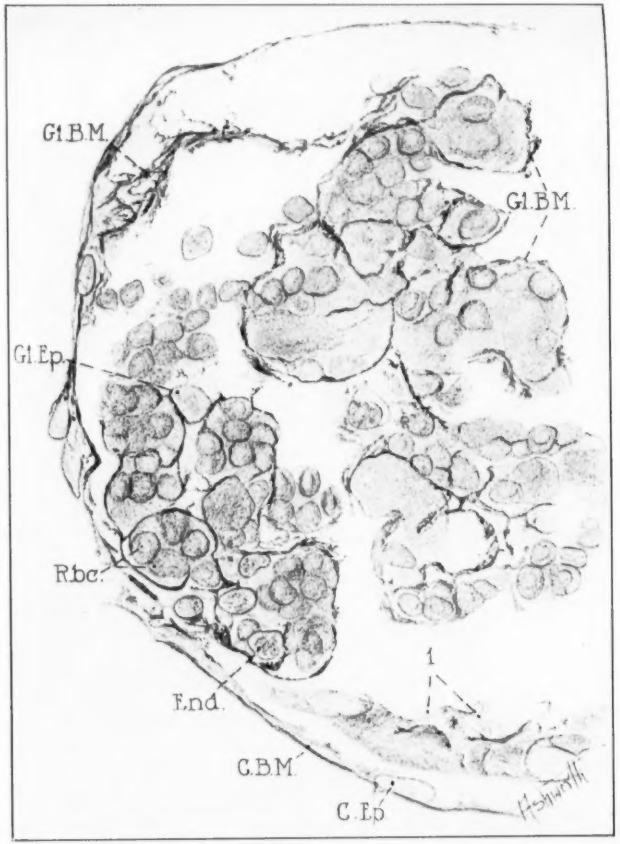

6

Hunter and Roberts

Glomerular Changes in Kidneys

HISTOLOGICAL STUDIES OF HYPERSENSITIVE REACTIONS *

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PART I

THE CONTRAST BETWEEN THE HISTOLOGICAL RESPONSES IN THE TUBERCULIN (ALLERGIC) TYPE AND THE ANAPHYLACTIC TYPE OF SKIN REACTIONS

Numerous irreconcilable differences between the experimental findings of Smith, Rosenau and Anderson, Otto and the innumerable other experimenters working with protein anaphylaxis, and those of the investigators of bacterial hypersensitiveness such as Römer, Baldwin, and Krause with tuberculin, Gay and Claypole with typhoidin, Fleischer, Myer and Shaw with abortin and Helmann and Kalning with mallein pointed to the existence of two distinct types of hypersensitiveness. Zinsser [1] was the first to emphasize and clearly to express the essential points of dissimilarity which may be presented in tabular form as follows:

	ANAPHYLACTIC TYPE	TUBERCULIN TYPE
Skin tests	Immediate Transitory	Delayed Prolonged
Serum	Antibodies demonstrable Passive transfer possible	Antibodies not demonstrable Passive transfer not possible
Results of intravenous injection	Acute shock	Delayed shock
Sensitizing antigens	Ordinary proteins Bacteria and some of their protein-containing products	Bacteria, best living but also killed if in condition to produce granulomatous tissue response
Testing antigens	Proteins Carbohydrate fraction of bacteria (apparently the most effective)	Bacterial proteins and protein-fractions only

One of us (Dienes)[2-7] in a series of studies has been able to support Zinsser's demonstration of an essential qualitative difference between the two types of hypersensitiveness. In this work, however,

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it became apparent that the bacterial nature of the antigen was not an essential determining factor in the development of the tuberculin type of allergy. It was possible to produce in tuberculous guinea pigs, with ordinary proteins such as egg-white or horse serum, a state of hypersensitivity characterized by delayed and prolonged skin reactions, an absence of demonstrable antibodies in the blood stream, failure of passive transfer, and insusceptibility to acute shock on intravenous injection. The best method to produce this type of sensitiveness was to inject the given protein directly into a tuberculous focus (a previously infected lymph node or testicle proved most convenient), and to test the animal within a period of three to ten days. The conclusions were drawn that in an infected animal a tuberculin type of hypersensitiveness to ordinary proteins could be produced, and that the distinctive characteristics of tuberculin sensitivity do not depend upon some special character of bacterial antigens or upon other secondary factors, but are the result of a special type of sensitization.

With such sharp immunological differences between the two types of hypersensitiveness it seemed only reasonable to expect that microscopic examination of the corresponding skin reactions should show clear-cut, recognizable differences. A survey of the literature, however, showed that no distinctive characteristics were generally recognized.

Descriptions of the histological features of anaphylactic reactions are found in the papers of Arthus and Breton,[8] Gerlach,[9] and Opie,[10] most of which, however, are based upon reactions produced in rabbits. They agree essentially upon inflammatory edema, polymorphonuclear infiltration and necrosis as the characteristic findings. Gerlach also studied reactions in guinea pigs, rats, dogs and the human skin. In these he obtained somewhat slighter reactions (though his antigen doses were still very large), in which serous and polymorphonuclear infiltration predominated and necrosis was less obvious or absent, but the results were essentially similar to those found in rabbits. He described one experiment with two guinea pigs tested 4 and 6 days after sensitization which showed at 48 hours a predominantly mononuclear infiltration. This corresponds closely to findings of our own which will be described in the second part of this paper, though Gerlach made no comparison between it and tuberculin reactions.

The older microscopic studies of the tuberculin reactions are somewhat contradictory. Most observers described it as a strong exudative reaction essentially like the Arthus phenomenon and minimized the difference of the anaphylactic and tuberculin types of hypersensitiveness (Spehl,[11] Auché and Augistrou [12]). In the German literature there are many claims that the tuberculin reaction reproduces the specific features of the tuberculous lesion (lit. see Blumenberg [13]). These claims are based upon the study of the late stages of skin reactions, several days to weeks old. The first stage of the reaction was regarded as an acute inflammatory response and received little attention. More recently Zieler and Hamel,[14] the main proponents of the histological specificity of the tuberculin reaction, in a survey of the conflicting claims admitted that the microscopic structure of the reaction showed no sufficiently distinctive characteristic to differentiate it from the late stages of nonspecific inflammatory processes (such as those following injection of *B. coli* vaccine), and that the only specificity of the tuberculin reaction lies in the fact that tuberculin produces its effect only in tuberculous animals.

The closely allied problem of reinfectious lesions will be discussed elsewhere.

The failure of previous investigators to find significant differences between the two types of hypersensitivity might well be explained: (1) by their choice of experimental animals, since rabbits rather than guinea pigs were usually used; (2) because strong reactions only were studied in which necrosis and the reaction to it dominated the picture, and (3) because the tuberculin reaction was not studied in the first few hours of its development when its special characteristics are most apparent.

We decided to reattack the problem, making use of the methods of sensitization developed by Dienes in his former studies. We have devoted our attention particularly to reactions of moderate and even slight intensity, finding in the course of our study that in these weaker reactions the differences between the two types were most readily observed. Two procedures were available for producing such reactions, both of which we have made use of: (1) the testing of highly sensitive animals with minimal test doses, and (2) the testing of very slightly sensitive animals with larger doses.

In the pursuit of this second method the significance of the early

reactions obtained within a few days of sensitization became more and more apparent and they were eventually intensively studied, the results appearing in the second part of this paper. The study of the slight reaction, moreover, not only offers a technical advantage but also gives us more information concerning the probable rôle of hypersensitization in the economy of the organism. Under natural conditions hypersensitive tissues come in contact only with small doses of an antigen, or if exposed to larger doses, as in certain infectious lesions, they are probably desensitized.

The following types of skin reactions have been studied histologically:

1. Tuberculin reactions
   (*a*) True tuberculin reactions.
   (*b*) Tuberculin type reactions produced with egg-white or horse serum.
2. Anaphylactic reactions
   (*a*) Passive.
   (*b*) Active.

The experiments have been carried out in both normal and tuberculous animals. Guinea pigs, for the most part, were used though in some experiments a small confirmatory series was run in rabbits. In the studies upon tuberculous animals the possibility of a nonspecific effect upon the histological picture was borne in mind. Kaufmann,[15] for instance, found that the blisters produced by cantharidin in tuberculous individuals showed a higher proportion of mononuclear phagocytes in the exudate than similar blisters produced in normal individuals, and Sabin *et al*,[16] Medlar,[17] and other investigators have noted the less directly pertinent increased mononuclear count in the peripheral blood in certain stages of tuberculosis. Indeed Selter and Tancré,[18] who noted the increased inflammatory response of tuberculous animals to many stimuli, regarded the tuberculin test itself as merely an intense form of this tendency of tuberculous animals.

Technique: The animals used throughout the experiments have been so far as possible light colored, fully grown males. Areas have been prepared for injection by careful shearing or plucking. No depilatories have been used. Several skin tests of varying age were often made upon the same pig without any noticeable desensitization. The animals were then killed, the appropriate areas cut out,

pinned upon corks, fixed in formalin and embedded in paraffin. Several sections were prepared from each block beginning at the center of the reaction and working outward at 2 to 3 mm. intervals. The sections were stained with hematoxylin and eosin.

Controls: In normal animals an intracutaneous injection of normal saline alone will produce a definite reaction. In the track of the needle there is a slight, rapidly developing necrosis and transitory polymorphonuclear infiltration. It is almost wholly limited to the needle track, however, and the surrounding tissue — the zone particularly studied in our experiments — shows only the faintest trace of polymorphonuclear and mononuclear infiltration. Egg-white, horse serum and synthetic tuberculin in the doses used show no more.

In many tuberculous animals the slight injury caused by the injection of normal salt solution or of egg-white or horse serum caused a much more definite reaction with edema and some initial polymorphonuclear infiltration. By 24 hours there was a slight but well marked predominance of mononuclears and the reaction was sometimes indistinguishable from a minimal tuberculin response. The variations between individual animals were sufficient to make it desirable to examine a control of NaCl or indifferent serum in each animal tested. Under these conditions no difficulty was experienced in judging the non-specific element of the reaction. As a further control of the effect of the tuberculous infection turpentine was injected intracutaneously into both normal and tuberculous animals. In the former an intense polymorphonuclear infiltration occurred. In the latter this was quite as intense but a slight increment of mononuclear infiltration was noted. The tuberculous infection evidently in no way inhibited the leukocytic exudation.

The Histology of the Tuberculin Reaction

The gross and microscopic appearances of tuberculin reactions vary enormously with the degree of sensitivity of the animal, its general condition, and the size of the test dose. A typical, moderately strong reaction might be expected to show the following gross changes. The bleb caused by the injection disappears in a short time. At 3 to 6 hours there is a slight, firm swelling with accompanying redness. At 12 hours a central necrotic spot begins to be apparent. By 18 to 20 hours a circumscribed violet (hemorrhagic) center 0.5 to 1 cm. in diameter is seen surrounded by indurated white and

reddish rings 2 to 3 cm. in diameter. At 48 hours the central zone is yellow-brown in color, the surrounding ring entirely red. From 72 hours on the brownish area dries up, forming a scab which eventually sloughs and falls off. The interval before complete healing is very variable.

In very slight reactions only a red area of induration with some central blanching may be made out grossly. By increasing the test dose in animals whose degree of sensitization is slight, extensive reactions up to 4 cm. in diameter may be obtained which show no necrosis. In contrast, well marked necrosis may be obtained in highly sensitive pigs with a dose so small that a lesion only 1 cm. in diameter is produced. The development of necrosis is therefore independent of the size or extent of the lesion and must depend upon some difference in the reaction mechanism.

An experience with the microscopic appearances of several hundred tuberculin reactions may be described in the following composite picture. As early as 2 hours after injection of the tuberculin, before any trace of reaction is visible grossly, the fixed tissue cells of the corium, both endothelial and fibroblastic, have become more prominent than normal and there is a well marked infiltration of large mononuclear phagocytes, with practically no admixture of polymorphonuclears.

At 6 hours the mononuclear infiltration is much more marked, appearing in part diffusely but being most evident in focal collections situated in the adventitia of small vessels and nerves. Polymorphonuclears may be present in moderate numbers, reaching in extreme instances 40 per cent of the invading cells, but more commonly comprising not over 10 to 15 per cent of the total. Evidence of necrosis cannot usually be made out up to this time. The absence of edema is quite striking in comparison with anaphylactic tests.

From 7 to 12 hours, in the stronger reactions, an alteration in the staining of the cells of the basal layer of the epidermis becomes apparent. The nuclei may become pyknotic. The cytoplasm is less strongly basophilic and often becomes vacuolated. A zone of polymorphonuclear infiltration appears just beneath this degenerating layer.

By 24 hours the necrosis of the epithelium is usually obvious and extensive. In the less severe reactions evidence of necrosis is sharply limited to this layer and a narrow zone of underlying derma. In the

more severe reactions a hemorrhagic type of necrosis of the underlying connective tissue may be fairly extensive. At this time the mononuclear predominance in the formula of the invading leukocytes is much less marked, and the polymorphonuclears may rise to 60 to 70 per cent of the invading cells. The mononuclears persist in large numbers, however, and even at this stage are far more numerous than at any period in the anaphylactic type of reaction.

By 48 hours regenerating epithelial cells can be seen growing in from the edges of the lesion beneath the necrotic remnants of the original epithelium, but above the underlying zone of connective tissue infiltrated with leukocytes. The polymorphonuclears are now sharply restricted to this zone and the deeper and peripheral parts of the lesion show an almost purely mononuclear infiltration. A considerable proportion of the polymorphonuclears are beginning to show evidences of degeneration, including fragmentation. The mononuclears on the other hand appear relatively uninjured and many of them contain phagocyted débris of the necrotic polymorphonuclears. In many the cytoplasm is somewhat swollen, more acidophilic than in the earlier stages, and more suggestive of the "epithelioid" appearance of the cells in true tubercular lesions.

In summary then, in the average tuberculin reaction of moderate intensity, a wandering cell infiltration predominantly mononuclear in character occurs. The mononuclear predominance is greatest in the early stage, up to 6 hours, and again in the late stages from 48 hours on. During the intermediate stage from about 7 to 48 hours the proportion of polymorphonuclears increases but rarely becomes predominant. During approximately this same period signs of degeneration and necrosis of the epithelium occur and the zone of polymorphonuclear infiltration is most intense in the neighborhood of the degenerating epithelial cells.

In less severe reactions, either in highly sensitive animals tested with a very small dose, or in recently inoculated animals in which the sensitiveness is not fully developed, the mononuclear predominance is still more marked at all stages up to 48 hours. In these reactions evidence of necrosis can rarely be made out.

The findings suggest, but are not sufficient absolutely to prove, that the polymorphonuclear infiltration evident in the middle period of the moderately strong reactions is in part, at least, secondary to necrosis of epithelium.

Tuberculin Type Lesions Produced with Egg-White

Tuberculous animals sensitized according to the method of Dienes by injecting a protein such as egg-white or horse serum directly into a tuberculous focus showed on skin test 3 to 10 days later a type of skin reaction corresponding in every detail of gross and microscopic appearance with the usual tuberculin tests described above. There was the same mononuclear predominance up to 6 hours, and again after 48 hours, with an intermediate period of relative polymorphonuclear increase at approximately the same period as the development of epithelial necrosis.

Since strong reactions of this type could be produced only in tuberculous animals it was obviously necessary to bear in mind the non-specific tendency of tuberculous animals to react to all inflammatory stimuli with a heightened mononuclear response. The degree of mononuclear infiltration in our test animals was, however, far greater than that observed in the non-specific control reactions. Moreover the further experiments recorded below and also those in Part II serve as added controls. In these we will show that an anaphylactic response produced in a tuberculous animal is characterized by only a slight mononuclear increase over the reaction produced in normal animals, and also that in non-infected animals a typical tuberculin type of reaction, albeit a weak one only, can be produced which shows particularly clearly the mononuclear predominance.

Anaphylactic Type Skin Reactions

For reasons which will become evident in the second part of this paper skin tests upon passively sensitized animals present a sharper histological contrast to the tuberculin type of reaction than do tests upon actively sensitized animals. To emphasize this contrast the passively sensitized animals will be discussed first.

Anaphylactic Type, Passive Sensitization

Technique: The animals, guinea pigs and rabbits, were passively sensitized with strong homologous sera, anti-egg-globulin in the case of the pigs, anti-egg-white in the case of the rabbits, and skin tested with 0.1 mg. a few days later. Egg-globulin was selected for some

of these tests because various proteins show relatively different powers of producing one or the other type of sensitivity. Egg-albumin, for instance, more readily produces the anaphylactic type, egg-globulin the tuberculin type. Therefore an anaphylactic type of reaction produced with egg-globulin is especially significant in demonstrating that the method of sensitization is more important than the chemical nature of the antigen. The egg-globulin preparation was of sufficient purity so that no precipitate was obtained with egg-albumin serum and that it failed to produce shock in animals sensitized with egg-albumin. The ovomucin, however, was not removed.

In such passively sensitized animals skin tests produced a rapidly developing wheal reaching in 1 hour 16 to 20 mm. in diameter with a peripheral pink or red zone of variable width. The reaction persisted up to 6 hours with but slight diminution in intensity, then faded quickly away. At 24 hours no trace remained except for the pin-point scar of the needle prick.

The microscopic appearances were quite different from those of the tuberculin tests. At 1 hour the blood vessels were dilated, the corium showed a marked diffuse edema, and polymorphonuclears were scattered in large numbers throughout the tissue. No increase in mononuclear elements could be made out.

At 6 hours the congestion and edema had slightly decreased. The polymorphonuclear infiltration on the other hand was more dense and a small number of large mononuclears had appeared. At 24 hours almost no trace of reaction was left except for a few scattered mononuclears.

In normal rabbits passively sensitized with anti-egg-white serum the gross reaction with 0.1 mg. of egg-white was less obvious and somewhat slower in development. By 6 hours, however, a small, soft, swollen, reddish spot was evident, which had decreased at 24 hours to a trace of redness. Microscopically the 6 hour reaction showed marked edema and dense infiltration with characteristic pseudo-eosinophilic polymorphonuclears and small numbers (5 to 10 per cent only) of mononuclears.

In the tuberculous animals similarly treated no gross difference in the reactions could be made out except that the local scar of the needle puncture was a trifle more noticeable and more persistent. Microscopically a slight increase of mononuclear leukocytes (not

above 15 per cent) was noticeable at 1 and 6 hours, but at 24 and 48 hours a definite mononuclear infiltration was evident. This, however, was no more marked than that of the salt solution controls.

Anaphylactic Type, Active Sensitization

Technique: The two animals used in these experiments were tuberculous. Egg-white was injected first into the tuberculous lesions and 6 days later 15 mg. were given intravenously. On subsequent skin tests they showed strong reactions of the anaphylactic type, whereas another group similarly treated, except for the intravenous injection, showed the tuberculin type of reaction. Two months later, after repeated skin tests, they were retested with 0.01 mg. ($\frac{1}{10}$ the usual dose) and the reactions were examined at 1, 6, 24 and 48 hours.

Grossly both pigs showed at 1 hour a wheal 16 mm. in diameter which increased to 22 mm. at 6 hours, then rapidly faded to a trace of redness without swelling at 24 hours.

Microscopically at 1 hour there was marked edema and a strong infiltration with polymorphonuclears, both diffuse and in clusters in the corium. The proportion of mononuclears was very small.

At 6 hours the edema and polymorphonuclear infiltration were still more marked. The proportion of mononuclears was slightly greater but still small in proportion to the number of granulocytes. No morphological evidence of necrosis was observed.

At 24 hours the edema and polymorphonuclear infiltration had largely disappeared, except for a small central zone in the traumatized area. A diffuse, fairly marked, predominantly mononuclear infiltration of the loose subcutaneous tissue was present, which did not, however, show the closely packed accumulations of mononuclears found in the tuberculin type reactions.

In other experiments reactions macroscopically and microscopically similar were observed in non-infected, actively sensitized pigs.

Summary and Discussion

The gross and histological pictures of the skin reactions in the anaphylactic and tuberculin types of hypersensitivity have been described and contrasted. The differences were found to be particularly emphasized in reactions of slight intensity on the one hand, and

in animals with relatively low sensitivity on the other. At all stages, however, recognizable differences between the two types occur, and these differences must be considered qualitative not quantitative.

The tuberculin type of reaction, whether produced with tuberculin or, in an appropriately treated animal, with a protein such as egg-white or horse serum, is characterized by the slow development of exudative phenomena and the early infiltration of mononuclear phagocytes. At a later period, from 12 to 24 hours, necrosis, most marked in and sometimes selectively localized to the epithelium appears. At the same period a marked increase in polymorphonuclear infiltration occurs, the temporal concurrence and the localization to the neighborhood of the degenerating epithelium suggesting that the leukocytic invasion may be primarily a reaction to necrosis. In tuberculin tests upon animals of relatively low sensitivity necrosis may not develop even in extensive reactions produced with large doses. In such reactions polymorphonuclear infiltration also remains slight.

In passively produced anaphylactic type reactions is found the sharpest contrast to this picture. In such reactions a rapidly developing edema, quickly followed by a fairly intense polymorphonuclear infiltration are the first phenomena observed. This reaction reaches its height somewhere between 1 and 6 hours and then rapidly fades away. At 24 hours a few persisting mononuclears are the only trace of the reaction.

In passive anaphylactic reactions produced in tuberculous animals a slight increase in the proportion of mononuclear phagocytes is noted. This is, however, no greater than the mononuclear reaction produced by indifferent sera or by salt solution in the same animals and does not significantly affect the marked polymorphonuclear predominance in the formula of the invading wandering cells.

In actively produced anaphylactic reactions, whether in normal or tuberculous animals, there is again a slight infiltration with mononuclears, evident at 6 hours and predominant at 24 hours. Their number, however, remains far below that seen in tuberculin type reactions and they fail to show the perivascular and perineural clusters so characteristic of that type.

Part II of this paper will present evidence for believing that in actively sensitized animals a certain amount of the tuberculin type of hypersensitiveness may develop along with, or persist as an un-

derlying factor in the stage of anaphylactic hypersensitiveness, so that what we are observing is really a mixed reaction with a predominance of the anaphylactic type.

Necrosis, though it occurs in the stronger anaphylactic type of reactions as described by Arthus and Opie, was not obvious in our experiments since the doses were purposely kept low. When it occurs it is, according to the observations of Opie and Gerlach, most marked in the fibroblastic and endothelial elements rather than in the epithelium. In tuberculin sensitivity, on the other hand, necrosis is the rule if the animal be highly sensitized even when the test dose is very small. Though in severe reactions extensive necrosis of all tissue elements may occur, in the slighter reactions it seems to be more or less selectively limited to the epithelium.

In this connection the results obtained by Aronson [19] in tissue cultures are of interest. He found that cultures prepared from tuberculous animals were killed by the addition of tuberculin, whereas cultures prepared from anaphylactically sensitive animals were uninjured by the addition of the appropriate antigen.

In the tuberculin type of hypersensitiveness the contact of cells and antigen starts an entirely different biological process from that produced in the anaphylactic type.

Conclusions

A histological comparison of the skin lesions of the anaphylactic and the tuberculin types of hypersensitiveness under appropriate conditions permits us to add to the already recognized differential points: (1) an initial hyperemic and exudative phase in the anaphylactic type contrasted with an initial phase of cellular infiltration in the tuberculin type; (2) a tendency to a predominantly polymorphonuclear leukocyte infiltration in the anaphylactic, compared with a predominantly large mononuclear infiltration in the tuberculin type, and (3) a tendency to selective epithelial necrosis in the tuberculin type, not observed in the anaphylactic type.

Part II

THE TUBERCULIN (ALLERGIC) TYPE OF RESPONSE IN THE FIRST PERIOD OF SENSITIZATION AND ITS RELATION TO THE PROCESS OF IMMUNIZATION

In the introduction to the first part of this paper we emphasized the importance of studying relatively mild reactions in order to obtain the sharpest differentiation between the anaphylactic and the tuberculin types of reaction. One of the methods used was the study of early skin tests, shortly after sensitization, when hypersensitivity was as yet incompletely developed. The study of these early reactions, moreover, served not only to emphasize the differences in histological reaction in the two types of hypersensitivity, but to throw an entirely new light upon the significance of the tuberculin type of hypersensitiveness by showing that it is a usual and perhaps universal stage in the immunological response of the organism to foreign protein injection.

When animals, tuberculous or uninfected, are tested with ordinary protein antigens within a short period (3 to 6 days) of sensitization, specific skin tests are obtained which show all the essential criteria of the tuberculin type of hypersensitivity. This has been pointed out before by Dienes in the case of appropriately treated tuberculous animals in which the gross appearances of the reactions are characteristic enough to determine the nature of the hypersensitivity. In the case of uninfected animals such strong reactions are never obtained. Grossly there is only the delayed development of slight hyperemia and induration which persists for 48 to 72 hours. On microscopic examination these reactions show the same mononuclear predominance described in our first paper as characteristic of the tuberculin type of sensitivity, and with this additional criterion we feel that there can be no doubt as to their classification.

Our experiments fall into two groups, the first performed upon tuberculous animals, the second upon uninfected ones. The tuberculous pigs were prepared by intraperitoneal infection and subsequent injection of the protein antigen into the peritoneal cavity — a method equivalent to direct injection into a tuberculous lesion. The rabbits were inoculated directly into a tuberculous node in the

groin in the usual fashion. The uninfected guinea pigs were sensitized intraperitoneally. Skin tests in all instances were done at short intervals, from 3 to 6 days after sensitization.

Experiment I

Tuberculous guinea pigs sensitized with egg-white, tested on the third and fourth days.

Table I

2 Hour and 24 Hour Readings of Skin Tests Performed on Tuberculous Guinea Pigs on 3rd and 4th Days after Sensitization

Guinea pig No.	March 13	March 16		March 17	
	Sensitization	Skin tests with 0.2 mg. egg-white i.c.		Skin tests with 0.2 mg. egg-white i.c.	
		2 hr. reading	24 hr. reading	2 hr. reading	24 hr. reading
308	3 mg. egg-white i.p.	Tr. white	20 × 20 red tr. sw.		
311	"	Neg.	12 × 12 tr.		
309	"			Tr. red and sw.	Sl. sw.[1]
314	"			Tr. red and sw.	12 × 21 bright red
307	Unsensitized control			Tr. red	Tr. red
310	"			Tr. red	10 × 10 tr.[2]

In this and the following tables the numbers signify mm., sw. = swelling, sl. = slight, tr. = trace.

All animals had received on March 5 (8 days before sensitization) 20 mg. R1 tubercle bacilli intraperitoneally.

[1] Guinea pig had a dark skin obscuring redness.
[2] Site of injection scratched.

Histology of Skin Lesions: The reactions observed were typical of rather mild tuberculin reactions, with a strong mononuclear infiltration, very few polymorphonuclears, no obvious necrosis. The appearances do not differ qualitatively from those observed in uninfected animals but are quantitatively distinctly more intense. The unsensitized tuberculous animals used as controls showed very slight infiltration which was predominantly polymorphonuclear in character.

Experiment II

Tuberculous rabbits sensitized with egg-white and tested 3 and 5 days later.

Table II

2 Hour and 24 Hour Readings of Skin Tests on Tuberculous Rabbits Sensitized with Egg-white 3 and 5 Days after Sensitization

Rabbit No.	Skin tests on 3rd day		Skin tests on 5th day	
	3 hr. reading	24 hr. reading	3 hr. reading	24 hr. reading
165	Neg.	16 × 13 tr. red		
167	Neg.	16 × 13 tr. red		
164			Neg.	28 × 23 bright red
163			Neg.	Small whitish
Controls			Neg.	Neg.

The four rabbits had been twice injected in the groins with the R1 tubercle bacillus strain. When a large swelling appeared 2.5 mg. of egg-white were injected into the swollen tissue. The dose used for skin testing was 0.5 mg.

In other experiments, to be fully described elsewhere, tuberculous rabbits showed no skin reaction 2 days after sensitization, but a well marked reaction in 4 days.

The microscopic findings in these rabbits were essentially similar to those of the guinea pigs, a marked mononuclear infiltration being the predominant feature.

Experiment III

Uninfected guinea pigs sensitized and tested on the sixth day with egg-white or horse serum.

Microscopically these lesions show at 6 hours, the time at which they first become macroscopically visible, a marked infiltration with mononuclear cells. This is particularly marked in the immediate subepithelial layer and also in the loose tissue between the corium and the muscularis. Polymorphonuclears may be almost absent or present in moderate numbers, the usual formula being about 80 to 90 per cent mononuclears to 20 to 10 per cent polymorphonuclears. In rare instances the latter may rise almost to 50 per cent but never

predominate. In the control reactions the infiltration is very much less extensive and polymorphonuclears make up 60 to 80 per cent of the invading cells.

TABLE III

4 Hour and 24 Hour Readings of Skin Tests on Uninfected Guinea Pigs 6 Days after Sensitization with Egg-white or Horse Serum

Guinea pig No.	December 22	Skin tests December 28				
	Sensitization	Egg-white 0.2 mg.		Horse serum 0.005 cc.		Tuberculin
		4 hr. reading	24 hr. reading	4 hr. reading	24 hr. reading	24 hr. reading
68	5 mg. egg-white i.p.	Neg.	10 × 10 red	Neg.	Neg.	Neg.
69	"	Neg.	20 × 22 red	Neg.	Neg.[1]	Neg.
70	0.1 cc. horse serum i.p.	Neg.	Neg.	Neg.	12 × 10 red	Neg.
71	"	Neg.	Neg.	Neg.	14 × 12 red	Neg.[1]

[1] These animals showed a trace of redness about the puncture wound.
This experiment was twice repeated with similar results, both grossly and histologically. For histological purposes these animals were killed 24 hours after skin testing. In other similarly treated animals, not sacrificed, the area of induration and redness persisted 48 hours before beginning to fade. Delayed and prolonged reactions of this type have been obtained as early as the third day after injection.

At 24 hours the infiltration is much more intense and averages 85 to 90 per cent mononuclear. No necrosis was observed in these reactions except in the line of the needle puncture.

SUMMARY OF EXPERIMENTS

Experiments have been recorded describing the skin reactions in sensitized animals, both tuberculous and uninfected, in the earliest detectable stage of hypersensitivity. It has been shown that the skin tests of this first phase of allergy show all the essential characteristics, not of the anaphylactic type, but of the tuberculin type of hypersensitivity. They are delayed in appearance, showing nothing grossly before 6 hours; they persist 48 hours or longer, and microscopically they show a predominantly mononuclear cellular infiltration in contrast to the polymorphonuclear infiltration characteristic of the anaphylactic type. The reactions observed in uninfected animals do not differ qualitatively from those of tuberculous animals but are quantitatively distinctly less intense.

Discussion

In Part I of this paper we showed that a sharp difference was recognizable in the histological appearances of the skin reactions in the anaphylactic and the tuberculin types of hypersensitiveness. The former was characterized by a highly transitory but fairly intense serous and polymorphonuclear exudation, the latter by a slow and relatively persistent mononuclear infiltration. These differences were particularly sharp in the less intense reactions, the maximal contrast being obtained between a passively sensitized anaphylactic animal and an early tuberculin test upon an animal still in a low degree of hypersensitivity. It was noted that in actively sensitized anaphylactic animals the contrast, though still readily discernible, was not so sharp, since a slightly higher proportion of mononuclears was evident at all stages than in the passively sensitized animals and the lesion was a little less rapidly evanescent. We felt justified in concluding that the mononuclear-polymorphonuclear ratio could be added to the already known criteria of differentiation between the two types of hypersensitivity.

In tuberculous animals it had already been found possible to produce at will an anaphylactic or a tuberculin type of hypersensitivity to the same protein (such as egg-albumin, egg-globulin or horse serum) by suitable variations in experimental technique. By the third day after sensitization a slight but definite tuberculin type of sensitivity could be demonstrated which increased in intensity for several days. After a variable period of time, greatly influenced by the mode of sensitization, the stage of the tuberculous infection, subsequent protein injections even as skin tests, and other factors which will be discussed at length elsewhere,[20] the anaphylactic stage of hypersensitiveness may supervene. Whether or not a certain degree of persistence of the tuberculin type of hypersensitiveness occurs after the development of the anaphylactic type is uncertain, but is suggested by the higher ratio of mononuclears in the skin tests upon actively sensitized as against passively sensitized animals.

In the present paper skin tests upon uninfected sensitized animals performed in equally early stages of sensitization have been described and found qualitatively similar though quantitatively less intense. The delayed reaction, the prolonged character, the mononuclear predominance, the specific character, as proved by cross-

sensitization with different proteins, all are characteristic of the tuberculin type of sensitivity, and we feel justified in concluding that it is a manifestation thereof. The tuberculous infection, therefore, is not essential to the development of this type of allergy, but serves merely to intensify it and also under some circumstances to prolong it and put off or prevent the appearance of the anaphylactic type.

Tuberculin hypersensitiveness, as we observe it in the course of a tuberculous infection, represents a strong and persistent development of the first stage of the sensitization process made possible by the special conditions created in the organism by the infection.

In this early stage of sensitization antibodies cannot be demonstrated in the blood stream by precipitin tests or complement fixation. If circulating antibodies are involved in the mechanism of this type of hypersensitivity they must be of an unknown nature or else exist in the blood stream in some as yet undetectable form. It appears more probable that we are dealing with an altered tissue reactivity, as specific to the protein antigen arousing it as are the usual serum reactions.

The conception of the relation between the tuberculin type of hypersensitiveness and the usual protein sensitization developed above resembles closely the often expressed opinion that tuberculin sensitivity is due to antibodies fixed upon the cells, whereas in the anaphylactic type circulating antibodies are responsible. Bessau and Detering,[21] moreover, after showing that in children injected with horse serum marked skin sensitiveness may develop before antibodies appear in the circulation, expressed the opinion that this early stage of sensitization corresponds with tuberculin hypersensitiveness. Our conception is essentially in accord with theirs, but with animal experimentation it was possible to obtain more extensive evidence in its support.

Scattered observations entirely consistent with those we have reported are not infrequent. The finding by Gerlach [9] of predominantly mononuclear infiltration in two guinea pigs tested on the fourth and sixth days after sensitization has already been mentioned in the introduction to our first paper. Redfern,[22] moreover, described reactions in the human skin, produced by reinjecting horse serum after a relatively short interval into the original sensitizing site, which were characterized by an infiltration of round cells which he regarded as lymphocytes. Spehl,[11] in a description of the his-

tology of tuberculin reactions mentioned the early necrosis of the epithelium and also noted that at certain stages the infiltrating wandering cells were mostly mononuclear. Perhaps, also, the histological finding in reinoculation experiments with vaccinia virus of a mononuclear infiltration of the corium is evidence of a similar mechanism in the field of the virus diseases.

The significance of such a conception to immunological theory and the morphology of infectious lesions is evident. If a specific tissue response occurs as early as the third day after parenteral introduction of an antigen, a tuberculin type of allergy may play a rôle in the cellular response to a great variety of infectious agents.

It is interesting to recall in this connection that the four diseases in which this type of hypersensitivity has long been recognized — tuberculosis, glanders, typhoid and the Brucella infections — are all diseases in which mononuclear phagocytes, at least in certain stages, dominate the cellular response. The assumption has often been made in the past that the granulomatous nature of the infection might be the cause of the delayed type of hypersensitiveness shown in these diseases. The experiments described in this paper at least suggest a converse relationship, that a tuberculin type of hypersensitivity may be the determining factor in the mononuclear infiltration.

Conclusions

1. The tuberculin type of hypersensitiveness represents the first stage of the immune response to parenterally introduced protein antigen.

2. It occurs in uninfected as well as in tuberculous animals.

3. A tuberculous infection quantitatively increases it but does not alter it qualitatively.

4. It may be demonstrable as early as the third day after sensitization.

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DESCRIPTION OF PLATES

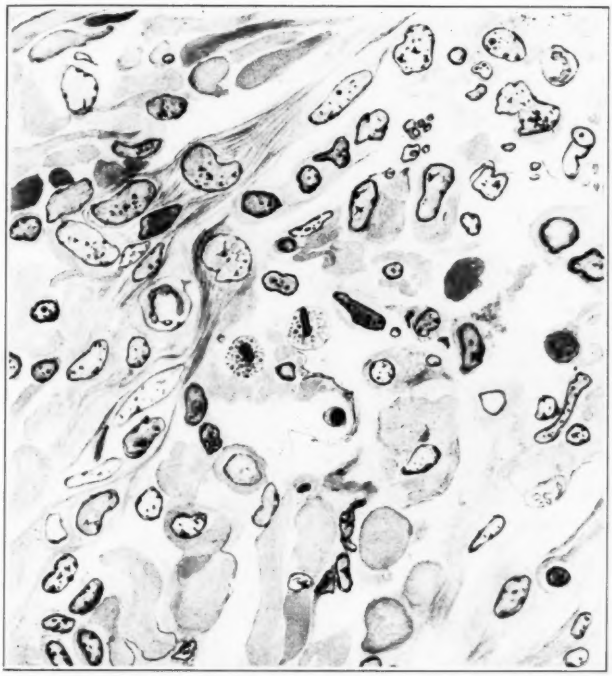
PLATE 112

FIG. 1. A tuberculin reaction of slight intensity produced on the fourth day after infection by the intracutaneous injection of 0.01 cc. of synthetic tuberculin. The lesion was excised 6 hours after the injection.

The reaction consists of a predominantly mononuclear infiltration most marked in the perivascular tissues. There is no apparent edema or necrosis.

FIG. 2. A tuberculin type of reaction produced with 0.2 mg. of egg-white in a tuberculous rabbit 4 days after sensitization by the injection of 5 mg. of egg-white directly into tuberculous lesions.

The morphological character of the lesion is in all respects similar to that of the true tuberculin reaction illustrated in Fig. 1.



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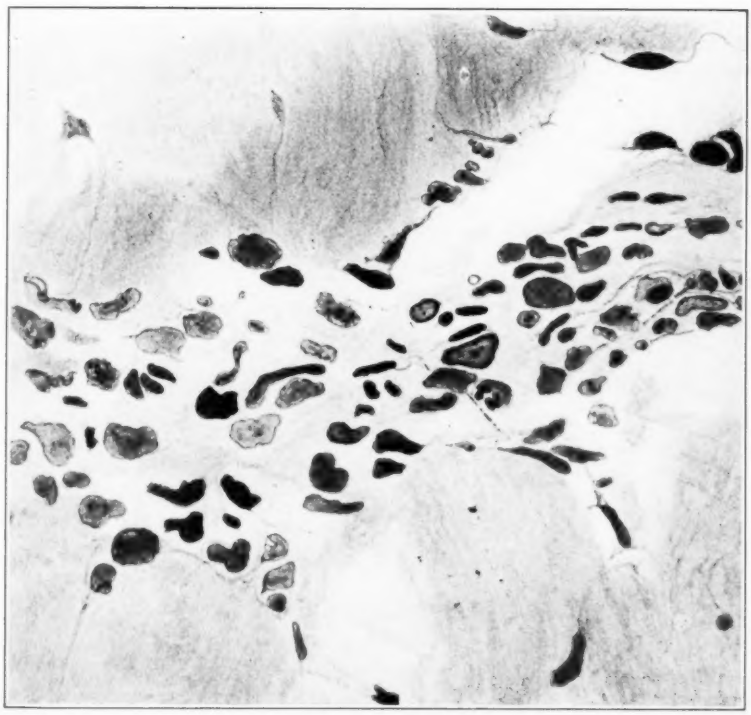

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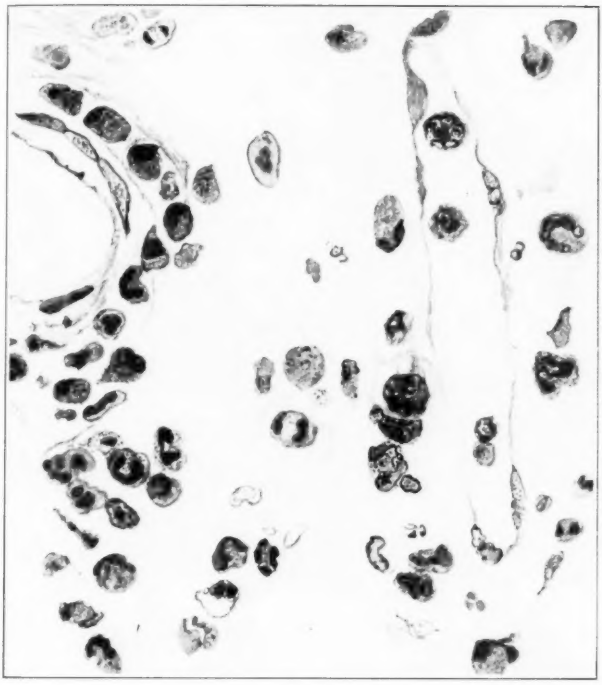
PLATE 113

FIG. 3. An anaphylactic type of reaction produced in a passively sensitized rabbit 6 hours after the intracutaneous injection of 0.2 mg. of egg-white.

The diffuse edema and the predominantly polymorphonuclear character of the invading leukocytes is evident.

FIG. 4. A reaction produced in an uninfected guinea pig by the intracutaneous injection of 0.2 mg. of egg-white. The pig had been sensitized 72 hours before by intraperitoneal injection of 2 mg. of egg-white. The lesion was excised 6 hours after the injection.

The mononuclear response, most marked in the perivascular tissues, is characteristic of the tuberculin type of sensitization.



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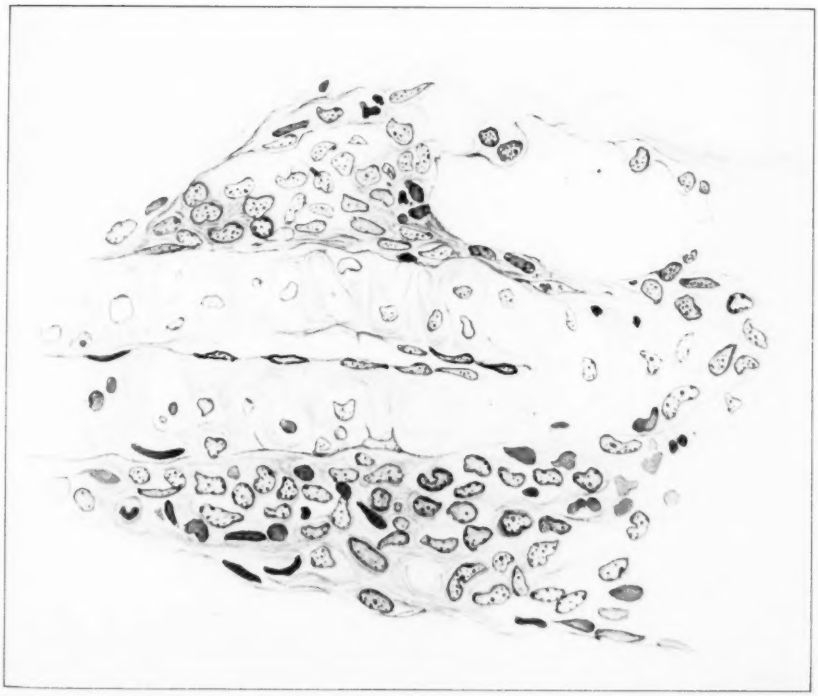

4

A HISTOCHEMICAL STUDY BY MICROINCINERATION OF THE INCLUSION BODY OF FOWL-POX *

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The inclusion bodies occurring in the epithelial cells of fowls injected with fowl-pox virus have attracted the attention of many investigators since their discovery by Bollinger.[1] Previous to 1900 many authors believed them to be microörganisms or parasitic protozoa. Later this hypothesis gave way to the theory that they were products of cellular degeneration due to the action of the virus.

One of the most important of the earlier contributions was that of Borrel,[2] who showed that smear preparations from the lesions revealed numerous, extremely small, coccoid-like bodies. Burnet,[3] two years later, confirmed this work and showed that in stained sections from the lesions these minute bodies and the inclusions occurred in the same cell. These findings led to the belief that the "Borrel bodies" were the causal microörganisms bound up within the cellular inclusions. This theory was upheld by many other authors but some still considered the inclusions (Bollinger bodies) to be simply a product of cellular degeneration. Among those who have supported the latter view are Ludford and Findlay,[4] whose interpretations, based upon a very detailed cytological study, were that the "Bollinger bodies" were a product of the reaction of the cell to the virus, rather than stages in the life cycle of an actual organism. They showed that the earliest stage in the formation of the inclusion bodies was a small vacuole, to the periphery of which minute granules were attached. The vacuoles then increased in number and size and in many instances coalesced to form one large inclusion body. These virus vacuoles, they pointed out, were enveloped by a lipoid sac and had an internal granular appearance. They further demonstrated the changes occurring in the mitochondria, which usually became

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† Aided by an appropriation from a grant made by the Rockefeller Foundation to Washington University for research in science.

vesicular and disappeared. The Golgi apparatus was fragmented and there was an extrusion of nucleolar material.

Woodruff and Goodpasture,[5] in a series of interesting experiments, isolated inclusion bodies from the epithelial lesions by tryptic digestion and were able to reproduce the disease by inoculation with a single inclusion body obtained by using Chambers' micropipette apparatus. They concluded that the inclusions were composed of minute bodies which represented the virus. Later these authors, by breaking up the inclusion body, demonstrated the infectivity of the "Borrel bodies," as compared with the non-infectivity of the lipoidal components of the inclusion.

Because of the possibility that viruses may be adsorbed onto the inclusion body, any information which can be obtained concerning the chemical nature of the inclusion should be of value. Furthermore, a knowledge of the chemical make-up of the inclusion may yield data concerning its origin from the various cell constituents. With this in mind, Scott [6] applied the technique of microincineration to the study of the nuclear inclusions found in the submaxillary glands of guinea pigs. Later, Covell and Danks,[7] while investigating the nature of the Negri body, made use of the same technique and found that the Negri body contains certain inorganic constituents. They suggest from these findings that the Nissl substance and the basophilic nuclear chromatin are principally concerned in the formation of the Negri body. The inclusion of fowl-pox being an extremely large one makes it favorable material for a chemical examination. It was decided, therefore, to apply this method to the inclusions of fowl-pox and ascertain what inorganic constituents, if any, they contain. The microincineration technique used was that devised by Policard [8] and more recently modified and applied to the study of cytological problems by Scott.[9]

Small pieces of tissue from 8 and 12 day lesions of fowl-pox were fixed in neutral formalin and absolute alcohol mixture (1 part of neutral formalin to 9 parts of absolute alcohol) for approximately 24 hours.* They were then dehydrated in several changes of absolute alcohol and embedded in paraffin. Serial sections were cut 4 or 5 microns in thickness and alternate ones were mounted on slides, using absolute alcohol as a floating medium and avoiding contact

* This material was obtained through the courtesy of Dr. C. Eugene Woodruff of Vanderbilt University.

with water. The excess of absolute alcohol was poured off and the sections allowed to dry. The slides were then placed one by one in an electric quartz tube oven and heated through a range of temperatures from about 40°C to 525°C for 25 minutes and, finally, gradually increased to 604°C over a period of 10 minutes. The slides were removed from the oven and cooled slowly. In order to protect the ashed remains coverslips were placed over them and the edges sealed with paraffin. The sections were then examined by means of a Zeiss cardioid condenser. The remaining sections of the series were mounted and colored with erythrosin-azur; these served as controls.

As can be seen from Figure 1, the control sections showed typical fowl-pox inclusion bodies. The lesions were quite far advanced, most of the affected epithelial cells were swollen and in some the nuclei had either disappeared or were represented by a small basophilic staining body at one side of the cell. Other less affected cells revealed the nucleus and the nucleolus quite clearly. Examination of a similar area of the incinerated sections demonstrated an inorganic residue in the inclusion bodies. The refraction of light from the deposits of mineral salts, both in the tissues and in the inclusions, made it impossible to photograph successfully details easily visible by direct examination of the preparations.

Under high power (oil immersion) the control sections, colored with erythrosin-azur, showed typical bodies, many of which consisted of a pink-staining outer area surrounding a clear irregular central area (see Fig. 2). This appearance was helpful in locating the inclusions in the incinerated tissues, as can be seen in Figure 3.

Cells of normal skin which have been incinerated show a considerable quantity of evenly distributed ash. The nucleus contains more mineral than the surrounding cytoplasm and stands out distinctly in the dark-field picture. By contrast the pathological cells show a notable decrease in the amount of both nuclear and cytoplasmic residue. The inclusion bodies themselves consist of a large aggregation of minute particles of grayish white ash (Fig. 4). Many cells show inclusions with this collection of small particles of ash around mineral-free, irregular central areas. This is depicted in Figure 3. Unfortunately the individuality of the particles of inorganic residue is obscured by refraction. These small particles of mineral ash apparently correspond with what are termed "Borrel bodies" both in relative size and in location. The nature of this ash is to some

extent revealed by the color of the oxides to which the inorganic constituents are reduced; free iron is represented by a reddish ash; calcium, along with other salts such as magnesium, leaves a grayish white ash; and organically bound iron, a distinctly yellow residue. The "Borrel bodies," therefore, presumably contain a large amount of calcium, as in the incinerated inclusions they are represented by minute particles which are grayish white in color.

Discussion

It is interesting that the findings in the Negri body and in the fowl-pox inclusion are the same in so far as there is a definite inorganic residue in both following incineration at high temperatures. This would suggest that the "Borrel bodies," like the Negri body, are perhaps products of degeneration of certain cell constituents, due to the action of the virus. It would appear from the results obtained by Woodruff and Goodpasture, who demonstrated the infectivity of "Borrel bodies," that the virus is so strongly combined with the inclusions that tryptic digestion and subsequent washings do not interfere with the union to any marked extent. This hypothesis is not unlikely, as it can be assumed that the "Borrel bodies" by virtue of their inorganic constituents might form a suitable substance for the adsorption of virus. That this is a possibility in the case of the Negri body and other inclusions is still to be proved by employing the technique of Woodruff and Goodpasture.

The incinerated sections of normal fowl skin show that the epithelium is rich in mineral ash, of which calcium forms a large part. The nuclei of normal nerve cells consistently show a small quantity of light brownish yellow ash, indicating the presence of masked iron; peculiarly enough, the nuclei of epidermal cells vary considerably in their visible content of this element. In sections of human skin, for example, the nuclei of some cells are devoid of visible iron while others adjacent to them possess it in relatively large amounts. With the available material it was impossible to determine whether the same holds true for the nuclei of normal skin cells of the fowl. It is conceivable that if a fundamental difference in chemical make-up exists in the nuclei, and perhaps also in the cytoplasm of epidermal cells, this might account for the affinity of certain cells for virus.

Although it can be seen that the nuclei of cells containing fowl-pox inclusion bodies show a decided decrease of inorganic salts, it could

not be concluded that this was the origin of the material forming the inclusion, as has been suggested for the Negri body. It is worthy of note, however, that these observations support those of Ludford and Findlay with regard to the extrusion of chromatin from the nucleus.

In 1930 Scott observed that the nuclear inclusion produced by virus of the submaxillary gland disease of guinea pigs left little or no ash after incineration. In view of these findings it is not entirely inconceivable that the nuclear inclusion represents a chemically different type of degenerative reaction than the cytoplasmic inclusion. Indeed, incinerated specimens of submaxillary glands of guinea pigs, possessing in the same cell both nuclear and cytoplasmic inclusions, do show such a difference. In this instance the cytoplasmic inclusions react the same as the Negri body and the fowl-pox inclusion — they leave a distinct grayish white ash residue after incineration.

There is in all probability a similarity in the nature of the shift of the mineral ash content of the nucleus and of the cytoplasm in nerve cells containing Negri bodies and in epithelial cells which have fowl-pox inclusions. Presumably this is due to the action of virus. With the evidence at hand it is impossible to suggest which cell constituents are responsible for the formation of fowl-pox inclusion bodies, although it is entirely probable that they are degenerative products of cell constituents — a result of the action of virus.

Conclusions

1. Following incineration at high temperatures the fowl-pox inclusion body leaves a grayish white residue consisting of minute particles of mineral ash. The location of this residue corresponds topographically to that part of the inclusion body which stains pink with erythrosin-azur.

2. The minute particles of mineral ash correspond in relative size and location to the "Borrel bodies" and are the inorganic residue of these structures.

3. There is evidence that the "Borrel bodies" having, as they do, a relatively large amount of inorganic material in them might well serve as a locus for adsorption of virus.

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DESCRIPTION OF PLATE

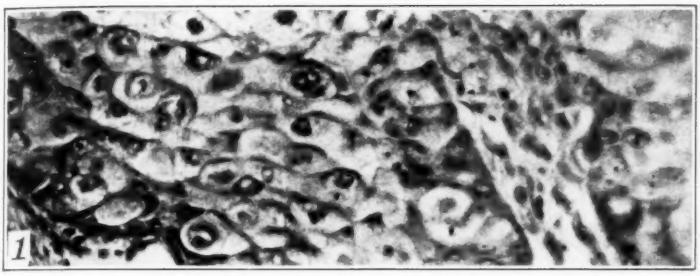
PLATE 114

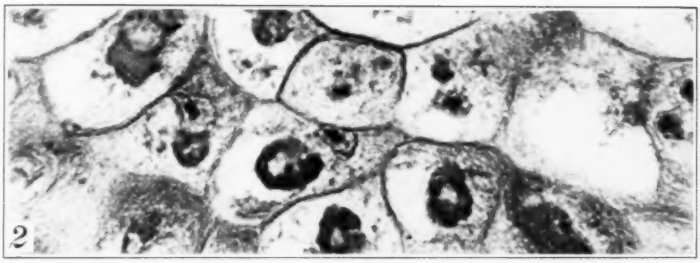
FIG. 1. Control section of skin lesions from chicken showing numerous fowl-pox inclusion bodies (erythrosin-azur). × 300.

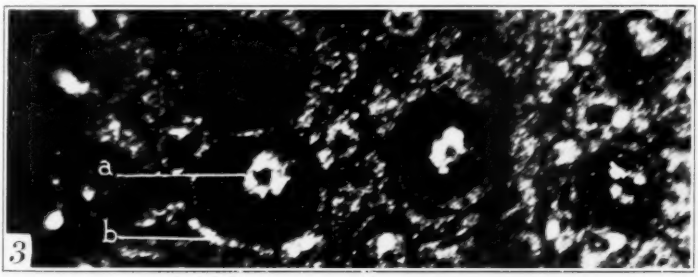
FIG. 2. Section from the same portion of tissue as Fig. 1, showing inclusions under high power. × 750.

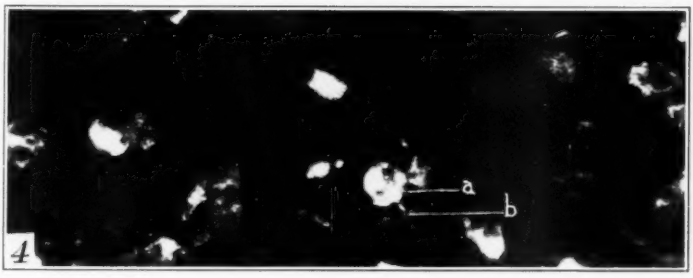
FIG. 3. Incinerated section showing typical inclusion bodies (*a*) lying within the cell boundaries (*b*). Dark-field illumination. × 750.

FIG. 4. Incinerated section under oil-immersion lens showing a cell containing a circular inclusion body (*a*) which reveals the particles of ash representing the residue of incinerated "Borrel bodies." The faintly outlined residue of the cell membrane (*b*) encloses the remains of the nucleus which is slightly to the left of the inclusion body (*a*). Dark-field illumination. × 750.









Danks Microincineration of Inclusion Body of Fowl-Pox

MEDIONECROSIS AORTAE IDIOPATHICA CYSTICA *

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The pathological changes leading to spontaneous rupture of the aorta in the absence of atheromatous or syphilitic lesions at the site of rupture remained vague until Erdheim's [1, 2] recent description of "Medionecrosis aortae idiopathica cystica." Rupture of the normal aorta incident to severe trauma has been recognized and accepted (Jores [3]) but the occurrence of spontaneous rupture of a normal aorta has remained a controversial question. Bostroem [4] was one of the first to describe such an occurrence and to consider the non-traumatic rupture of the normal aorta possible. Jores in reviewing the subject stated: "Spezifische für die Aortenruptur charakteristische Veränderungen sind nicht nachweisbar, ins besondere auch nicht an der Rupturstelle." Kaufmann [5] cited cases of his own and from the literature of spontaneous rupture in apparently normal aortas. Benda [6] called attention to the relatively insignificant histological changes seen in cases of spontaneous rupture. Karsner [7] doubted that a normal aorta is ever the seat of non-traumatic rupture. This latter opinion was shared by von Schnurbein, [8] who accounted for the non-appearance of demonstrable morphological change on the basis of the angiomalacia described by Thoma,[9] and by Oppenheim,[10] who tested the resistance of normal arteries to intravascular pressure *in vitro* and found that the pressure required to rupture them was higher than could possibly be attained *in vivo*.

Spontaneous rupture of the aorta has been reported in animals; and Krause [11] in a review of vascular disease in animals stated that it is not an uncommon occurrence in horses, and that although arteriosclerosis has been the cause in some, others have been studied in which histological changes were insignificant, or absent.

Erdheim's exhaustive study of two cases of spontaneous rupture of the aorta was prompted by the case reports of Gsell.[12] These ruptures occurred in the ascending arch as the result of medial necrosis, which was distinguished from arteriosclerosis and from syphilis. Additional cases have been reported by Cellina,[13, 14] Levinson [15] and

* Received for publication May 20, 1932.

Neubürger.[16] Another case described by Orsós-Debrecen [17] probably should be included in this group.* The necessity of preparing many sections from the region of the rupture was stressed especially by Erdheim, and in the two cases reported by Levinson the changes were so insignificant that they would have been missed in the course of a casual examination.

Recently three cases of spontaneous aortic rupture, without demonstrable inflammatory changes or significant intimal sclerosis at the site of the tear, have been observed in this laboratory. The general histological examination was made from sections stained with hematoxylin and eosin. The elastic elements were stained by Weigert's and Saphir's orcein methods and by a combination of the Verhoeff and the Masson trichrome light green stains. Smooth muscle was identified with the Van Gieson and the Masson trichrome stains. Fat was stained by Sudan III, scharlach R, Nile-blue sulphate and osmic acid. Calcium was demonstrated by the von Kossa test. Chromatropic material was stained by thionin, cresyl violet R, and polychrome methylene blue.

Case Reports

Case 1. † *Clinical History:* A white man, 50 years of age, collapsed while at rest and died before reaching the hospital. There had been no significant previous illness and his collapse had not been preceded by any unusual physical exertion. The clinical impression was "death from cerebral hemorrhage." The blood pressure was not known. No Wassermann had been taken.

An autopsy was performed and the pathological diagnoses were: rupture of the aorta with dissecting aneurysm of the ascending arch and perforation into the pericardium; hemopericardium (about 500 cc.); cardiac hypertrophy; arterial and arteriolar nephrosclerosis, severe; generalized arteriosclerosis; passive hyperemia of spleen and liver; pulmonary emphysema, severe.

Immediately above the left coronary and non-coronary cusps there was a transverse tear, 4 cm. in length, through intima and media. In association with this tear there was a dissecting aneurysm

* Just before receiving the printer's proof of this study, an article by Klotz and Simpson on spontaneous rupture of the aorta was published. They have described five cases of spontaneous rupture of aortas, the seat of patchy or diffuse medial necrosis, not accompanied by inflammatory reaction or intimal change at the site of rupture. They did not, however, describe fatty change as being a feature of the medial degeneration.

Klotz, O., and Simpson, W. Spontaneous rupture of the aorta. *Am. J. M. Sc.*, 1932, **184**, 455.

† The author is indebted to Dr. Harry Goldblatt for permission to publish this case.

of the entire ascending portion of the thoracic aorta, so that the separated intima and media lay as a partially detached tube within the adventitia, and separated from it by an extensive hematoma. In the beginning of the transverse arch there was another transverse tear through intima and media for about 1 cm., marking the site of the re-entry of the blood into the lumen of the aorta. Inferiorly the dissecting aneurysm had perforated the pericardium through a ragged defect, measuring about 2 cm. in length. The pericardial sac was distended by about 500 cc. of blood.

The heart weighed 450 gm. and presented no abnormality, other than the hypertrophy which was principally of the left ventricle. The aortic ring measured 7.5 cm. in circumference and the mid-portion of the ascending arch 8.2 cm. The ascending thoracic portion of the aorta was almost completely free from intimal change, there being only a few slightly elevated, irregularly outlined yellow plaques. Moderately severe intimal sclerosis was seen in the lower portion of the thoracic aorta, with increased severity in the abdominal portion.

The media in the region of the tear was thin, but it could not be determined macroscopically whether the thinning was actual, or due to splitting of the media by the dissecting aneurysm. Section through the media disclosed a mottling by small, gray areas, which were in places confluent so as to obscure the entire thickness of the usual yellow zone. No cysts could be identified on macroscopic examination.

Histological Examination: In multiple sections, both transverse and longitudinal, through the aorta at the site of the rupture, the intimal change was minimal. The internal elastic lamella was intact and irregularly thickened. The intima was smooth and exhibited no degenerative changes, other than the presence of homogeneous, faintly staining, basophilic intercellular substance. The subendothelial connective tissue was increased in amount, but not uniformly, so that the free surface was slightly undulating. There was some calcification along the internal elastic lamella and finely dispersed fat droplets in the homogeneous intercellular matrix.

The media was not uniformly thin, and had been split by the dissecting aneurysm which had separated the outside four or five lamellae. Generally throughout the media there was a faintly staining, homogeneous, basophilic substance between and around elastic

fibrils. This substance was situated between the muscle cells and the elastic fibrils and its presence often caused a swelling of the lamellae. Although the distribution was general, it was more abundant in the middle and inner third of the media. When stained with cresyl violet R and differentiated with dilute acetic acid (Schultz [18]) it stained rose or light red. A similar staining reaction was obtained with polychrome methylene blue. The interfibrillar amount of this chromatropic substance varied and with its increase there was a corresponding decrease in the interfibrillar cellular elements. Frequent areas were encountered in which swollen lamellae were identified only by the elastic fibrils, the muscle and connective tissue cells having completely disappeared. When the cellular elements of contiguous lamellae had been replaced by this chromatropic substance, damage to the intervening elastic fibrils was frequently manifest by defects and projecting spurs. When several lamellae had become confluent, cyst formation was observed and a continued accretion of chromatropic substance was suggested by the rounding of the limiting tissue. Such cysts frequently included portions of eight to ten adjacent lamellae and were seen most frequently in the middle and inner thirds of the media. They were not prominent in the immediate vicinity of the tear, and although the largest cysts were found in sections of the ascending portion of the thoracic aorta the chromatropic change and the formation of small cysts were found in the descending portion and in the abdominal aorta. In the abdominal aorta the change was most marked immediately beneath atheromas.

In the ascending thoracic aorta, repair of these defects by fibroblastic proliferation was prominent and various stages of healing were seen up to complete filling in of the defect by fibroblasts. This repair was accomplished without vascularization and at no stage in the process were exudative or phagocytic cells seen. The scars were striking in preparations stained for elastic tissue, because of the sharp delimitation produced by the free ends of elastic fibrils (Fig. 1). The newly formed connective tissue did not conform to the normal structure and the long axis of the cells was frequently oblique or transverse to the long axis of the smooth muscle cells of the aorta. Very fine, newly formed elastic fibrils were seen to form a network in the scars. Such scars were seen in all layers of the media and did not originate in the adventitia.

What was interpreted to be a secondary cystic degeneration of partly or completely healed lesions was seen in the form of reaccumulation of serous, rather than chromatropic, substance between fibroblasts, with a disappearance of cells and the reformation of fluid-filled defects (Fig. 1).

There was little, if any, compensatory adventitial thickening for the areas of medial damage. At the site of the tear there was a diffuse extravasation of erythrocytes throughout the adventitia. The vasa showed changes corresponding to those seen generally in arterioles, that is, simple intimal proliferation, without perivascular infiltration. The most severe arteriolar damage was present in the kidneys, where obliteration was frequent. In the adventitia, however, no obliteration was observed and vasa were not uniformly affected, many being unchanged.

No disseminated necrosis of the type described by Cellina was seen either in the aorta or its branches.

Fat was stained in the intima and in the media by scharlach R and Sudan III. In the media it was deposited in the form of small droplets in the chromatropic substance along the elastic fibrils. It was not increased with further destruction of lamellar cellular elements and was not present in the cysts. No anisotropic lipoids were seen with the polarizing microscope.

CASE 2.* *Clinical History:* A white man, 56 years of age, was admitted to the Cleveland City Hospital with dyspnea, precordial pain and profuse blood-tinged expectoration. The present illness had begun several days previously with a chill and expectoration of blood-tinged sputum, following which his ankles had become swollen. He was known to have had hypertensive heart disease upon a previous admission, at which time his blood pressure was 210/120.

Physical examination disclosed an acutely ill patient with a large active heart, dyspnea, cyanosis and auricular fibrillation. The cardiac embarrassment continued without change until the seventeenth hospital day, when he was seized with a sudden sharp pain in the precordium and left shoulder. He died six hours later with a clinical diagnosis of "probable coronary occlusion."

An autopsy was performed and the pathological diagnoses were: rupture of the aorta with dissecting aneurysm of ascending arch and perforation into pericardium; hemopericardium (1250 cc.); cardiac hypertrophy and dilatation; generalized arteriosclerosis; arterial

* The author is indebted to Dr. David Seecof and Dr. R. W. Scott of the Cleveland City Hospital for permission to publish this case.

and arteriolar nephrosclerosis; infarcts of kidneys; chronic passive hyperemia of lungs, liver, spleen and kidneys; pulmonary emphysema.

There was a transverse tear 2.5 cm. long through media and intima about 1 cm. above the non-coronary aortic cusp. The edges of the tear were sharp and undermined by a dissecting aneurysm which extended about 5 cm. above the tear, and below into the pericardial sac through a slit-like laceration 1 cm. in length. The margin of the tear did not show any evidence of healing and the blood between media and adventitia and within the pericardium showed no organization.

The heart weighed 925 gm. and the hypertrophy was preponderately of the left ventricle. The aortic ring measured 9.5 cm. in circumference and the ascending portion of the thoracic aorta 12 cm. The intima of the ascending thoracic aorta was thin and smooth, except for a few small yellow plaques, the largest being at the commissure between the right and non-coronary aortic cusps, and measuring about 1 cm. in circumference and 2 mm. in thickness. From the arch down, intimal sclerosis became increasingly more severe with atheromatous ulceration of the lower abdominal aorta. Section of the media near the point of rupture did not disclose any characteristic alterations in structure.

Histological Examination: The intima in the region of the tear was irregularly thickened by fibrous connective tissue, but without formation of atheroma. The subendothelial connective tissue was edematous and relatively anuclear. Below the transverse arch intimal sclerosis became more severe, with hyalinization, fat infiltration, formation of atheromatous plaques and ulceration, which extended well into the inner third of the media.

The media in the region of the rupture was thin and profoundly altered in structure. Chromatropic degeneration was focal and severe but cyst formation was not prominent. In the areas of such degeneration, the media appeared attenuated, as though it had stretched in a transverse direction, with elongation of the surviving muscle cells and breaking of the elastic fibrils. In evidence of this, there were entire low power fields in which only short, isolated fragments, or no elastic fibrils were seen. Such defects in the elastic tissue were occupied by parallel-disposed smooth muscle cells, which conformed in direction to the structural pattern of the media

(Fig. 2). These cells were separated by chromatropic substance and in consequence the tissue was loose. In other places, exhibiting a similar type of medial defect, the muscle cells were compactly disposed and free from degenerative changes, with occasional nuclei undergoing direct division. The nuclei in such areas were generally larger and more chromatic than in other portions of the media (Fig. 2).

In lesions of this sort the only demonstrable elastic fibrils were either the projecting spurs at the edges or isolated, short, thick fragments of the disrupted fibrils. No new network of elastica was apparent. The defects were principally in the outer third of the media, but in several places they extended completely through the media and included the internal elastic lamella. Their outline generally tended to be wedge-shaped, with the broad base of the defect toward the adventitia; but not infrequently the defect had parallel edges and extended radially, or obliquely from the inner to the outer portion of the media. They were not vascularized and were not associated with exudative cells.

Smaller defects in the media were cystic and apparently due to the confluence of lamellae, the seat of chromatropic degeneration, and in these fibroblastic proliferation was seen to effect repair. These fibroblastic scars differed from the larger defects filled by smooth muscle in that the arrangement of the connective tissue was irregular and without pattern, and in such areas degeneration was usually progressive so that cysts had developed. The contents of the cysts were serous, rather than chromatropic.

The same changes were seen, but with less severity in other portions of the aorta. In the abdominal aorta they were complicated by more severe intimal change, with corresponding medial damage related to it.

The deposition of fat was inconspicuous in the ascending thoracic aorta, and was in the form of fine droplets in the intima and near the elastic fibrils throughout the media. It was not increased in the cysts. In the abdominal aorta, large deposits of fat were observed in the intima and adjacent portions of the media. The fat stained blue with Nile-blue sulphate and red with scharlach R and Sudan III. It did not become impregnated with osmic acid. Finely granular deposits of calcium were demonstrated in the media, particularly in the chromatropic substance, by the von Kossa test.

The adventitial change was nowhere significant. Vasa vasorum were the seat of some intimal sclerosis, corresponding to the generalized arteriolar disease. In the region of the rupture, and around the hematoma, there was some organization with leukocytic infiltration.

CASE 3. *Clinical History:* A white man, 44 years of age, collapsed while at work and died five days later. During that period he complained of continuous, intense, precordial pain and his progress was characterized by increasing circulatory failure. The blood pressure and the blood Wassermann reaction were not known. His past history was not significant, other than that he had had rheumatism and influenza thirteen years before. The clinical diagnosis was "probable coronary thrombosis."

An autopsy was performed and the pathological diagnoses were: rupture of the ascending portion of the thoracic aorta with dissecting aneurysm into pericardium; hemopericardium (about 500 cc.); generalized arteriosclerosis, mild; hypertrophy and hyperplasia of thyroid gland.

There was an annular tear through intima and media of the aorta, which included about four-fifths of the circumference of the vessel and was situated about 3.5 cm. above the aortic ring. The maximum separation of the torn edges was 2 cm. Communicating with the tear was a dissecting aneurysm which extended up to the level of the innominate artery and down into the pericardium by means of multiple small lacerations. There was evident organization of the intramural hematoma. Just above the large tear, which had clean, free margins, there was a small parallel tear, the edges of which were approximated by what appeared to be a thin, fibrous cicatrix. The media just above the midportion of the large tear was very thin, and here as well as in other areas near the tear the continuity of media was interrupted by small cystic spaces and gray patches.

Histological Examination: In several longitudinal sections taken through the tear, the external portion of the media above and below it was anuclear and stained lightly with eosin. The junction between this anuclear zone and the adjacent non-necrotic media was sharp and devoid of any reactive changes. The affected tissue had the appearance of coagulation necrosis without exudation. It occupied about half the thickness of the media at the site of the tear and extended about 5 mm. above and below it. The elastic fibers remained unaffected and in sections treated with orcein, without a nuclear counterstain, the lesion could not be recognized. The inter-

fibrillar substance stained yellow with the Van Gieson technique and pale green with the Masson trichrome light green stain. This was the only site of this type of necrosis seen in the aorta. It was thought that this necrosis was directly related to the presence of a large intramural hematoma, which lay between adventitia and media, at this point. The hematoma showed extensive peripheral organization, and several vasa in this vicinity were occluded by recent thrombosis. Although the type of necrosis was similar in appearance to the disseminated medial necrosis described by Cellina, the apparent cause for the necrosis was ischemia, due to secondary thrombosis of the vasa incorporated in the large hematoma.

In a number of blocks taken transversely and longitudinally in the vicinity of the rupture, another type of lesion was seen which varied greatly in severity. In one transverse section just above the midportion of the tear only isolated rests of the original media could be identified. These were separated by avascular scars of more or less compactly arranged fibroblasts which frequently involved almost the entire thickness of the media. The scarring was most severe in the mid and outer third, and was not associated with exudation. The scars were not flame-shaped, as seen in syphilitic aortitis, the internal elastic lamella was intact and the overlying intima thin and unchanged.

Chromatropic substance was seen in varying amounts in the media of the aorta and of the pulmonary artery, but was most prominent in the ascending thoracic portion near the rupture. Generally its distribution was in the intima, and between muscle cells and elastic fibers of the media. In longitudinal section it appeared to encase the elastic fibrils. Near the rupture it had frequently replaced the muscle cells of one or several adjacent lamellae with frequent destruction of the intervening elastic fibrils to produce cystic defects. In the larger defects, one of which measured about 2 mm. in length by 1 mm. in thickness and whose transverse extent was not determined, the contents no longer stained specifically for chromatropic substance, but were serous. One such defect was very close to the tear and was filled with extravasated erythrocytes. Spurs and fragments of the original elastic fibrils projected into the defects and in those showing fibroblastic repair a newly formed fine mesh of elastic fibrils was seen. The largest cysts were seen in the middle and outer third of the media, although smaller ones were present in the inner third (Fig. 4).

Calcium was present in the form of finely granular deposits near the internal elastic lamella and occasionally in the chromatropic substance of the media. The presence of fat was prominent in the middle third of the media near the tear (Fig. 3). It was stained red by scharlach R and Sudan III and blue by Nile-blue sulphate, but was not impregnated by osmic acid. The fat was not anisotropic. It was distributed in the form of fine droplets in the chromatropic substance and occasional collections were seen in the poles of the nuclei of muscle cells. In other locations the fat was almost entirely confined to the intima, and here its distribution was scanty and irregular.

The edges of the rupture were covered with fibrin and exhibited organization, especially where the tear extended into the adventitia. The vasa, except immediately beneath the tear, showed no significant pathological change.

Discussion

In the three cases of spontaneous rupture of the aorta just described there were gross and microscopic medial changes which were most severe in the vicinity of the rupture and of such severity as to be considered responsible for the spontaneous tearing of the weakened wall. These changes were not associated with demonstrable inflammation or with significant intimal sclerosis, and in most respects were like those described by Gsell, Erdheim, Cellina, Levinson, and Neubürger in similar cases.

In all three cases there was an increase in homogeneous, pale staining, basophilic, acellular material in the media between the muscle cells and elastic fibrils. In longitudinal section this material appeared to encase the elastic fibrils. This substance was found generally throughout the aorta, but was more prominent in the ascending arch. It stained red with thionin and light red or rose with cresyl violet R and with polychrome methylene blue. It showed a distinct affinity for fat and in one case for calcium. Prolonged formalin fixation rendered the presence of only a small amount of calcium in the other two cases non-significant. The significance and origin of the chromatropic material has been studied exhaustively by Schultz.[18] Because of its property of staining like mucin, a group of investigators, including Hueck,[19] termed the process "Schleimige Degeneration," while Schultz preferred to designate the material as

a chromatropic substance without definite commitment as to its mode of origin. It is quite well agreed that this chromatropic matrix becomes more prominent in the intima and media of blood vessels with advancing age, and that it exhibits a definite affinity for fat and calcium. Whether it be physiological or pathological, it was regarded by Schultz as "the precursor of further degenerative processes."

Transverse and longitudinal sections of the aortas of seventy adults were studied as to the distribution of the chromatropic substance in individuals not dying of aortic rupture. The relative distribution in intima and media varied. In some it was inconspicuous in the media and prominent in the intima, and *vice versa*. In six of the seventy cases there were focal areas within the media where the entire interfibrillar substance of several contiguous lamellae was acellular and chromatropic, with disruption of the interposed elastic fibrils. Whether the discontinuity of the elastic tissue was artefact, as proposed by MacCallum,[20] or actual and indicative of angiomalacia as suggested by the work of Thoma,[9] it remains that medial defects were seen. In three of the six this medial damage was immediately beneath areas of atheromatous intimal change, and in the other three the medial lesions were not associated with more than mild overlying intimal sclerosis (Fig. 5).

Precisely the same sort of change that was seen in slightly less than 10 per cent of adult aortas, in which only two blocks from each aorta were studied, was present in the three cases of spontaneous rupture, but with greater severity. The medial damage varied from simple accumulation of chromatropic substance around the elastic fibrils to the formation of large, macroscopic cysts (Fig. 4). In the earlier stages the elastic fibrils remained intact while, as the lesion became larger, there was complete destruction of elastic fibrils, leaving short projecting spurs and isolated fragments. These defects were independent of atheroma, and although they were present in all portions of the media the largest cysts were seen in the middle and outer thirds. As the cystic character became manifest, the chromatropic substance lost its specific staining qualities and became serous. Whereas fat in the form of small droplets was found in the interstices between muscle cells and elastic fibers in the earlier stages of degeneration (Fig. 5), it was notably absent from the cysts. The fat stained with Sudan III, scharlach R and Nile-blue sulphate,

but was not impregnated by osmic acid. The fat was not entirely extracellular but was seen within the muscle cells, especially at the poles of the nuclei. Finely granular deposits of calcium could be demonstrated in the chromatropic substance by the von Kossa reaction, but as in the case of fat, it too disappeared in the cysts whose contents were serous.

These observations indicated that with age, the aortic media acquires gradually increasing amounts of perielastic chromatropic substance, as described in detail by Schultz, and that the medial change is not necessarily paralleled by equally severe intimal change. Furthermore, this degeneration may result in the formation of circumscribed medial defects, which in the case of spontaneous aortic rupture may attain macroscopic dimensions and apparently produce significant weakening of the aortic wall.

Damage of this sort to the media, without cyst formation, was seen especially in Case 2. Here, in focal areas of lamellar degeneration, the elastic fibers had torn and separated, leaving large portions of the media, sometimes involving its entire thickness, devoid of elastic tissue. In these areas degeneration was in places reversible, because smooth muscle hyperplasia occurred in attempted compensation for the weakening, and advanced lesions occupied by thickly disposed smooth muscle cells were often devoid of degenerative changes (Fig. 2).

The changes so far described have been qualitatively similar in cases of simple aortic sclerosis to those seen in spontaneous rupture. One phase of the process was, so far as our observations are concerned, peculiar to spontaneous rupture, and that was the healing of the foci of medial necrosis. The healing lesions have been described in great detail by Erdheim, whose observations were in accord with ours, except that we did not see regeneration of smooth muscle in the repair of cysts. Hyperplasia of smooth muscle cells was seen at the site of elastic tears (Fig. 2), but in our cases of spontaneous rupture it appeared that the cysts were repaired principally by fibroblastic proliferation (Fig. 1). This repair was accomplished without vascularization and without the presence of exudative cells. The scars marking the site of cyst formation differed from the defects created by elastic tearing in that in the former the pattern of the proliferated fibroblasts was irregular, while in the latter the

long axis of the smooth muscle cells conformed to the direction of the lamellae. As pointed out by Erdheim, the degenerative changes and cyst formation constitute a vicious cycle, which he likened to atrophic cirrhosis of the liver. The newly formed media after and during repair underwent degeneration with reformation of cystic spaces. The accumulation of chromatropic material was not a feature of the degeneration in new media, nor was fat infiltration or calcium deposition.

As has been stressed by Gsell, Erdheim, Cellina, Levinson and Neubürger, this type of medial necrosis can in all stages be differentiated from syphilitic mesaortitis. Significant vascular changes in the adventitia fail and there is no exudative reaction. In all of the cases so far described the medial changes were not part of an atherosclerotic process, the intima in the involved region being relatively free from change (Fig. 4).

The disseminated, non-cystic form of medial necrosis described by Cellina was not seen in these cases, although a secondary necrosis near the rupture in Case 3 stimulated this type of necrosis. Here, however, the necrosis was apparently due to secondary occlusion of the vasa vasorum by thrombosis, incident to the dissecting aneurysm. Cellina found this type of necrosis in nine of ten aortas of aged individuals examined. In these nine aortas he found 103 foci of acellular necrosis in which the elastic fibers were preserved. This same type of necrosis was observed by Erdheim and by Levinson in cases of idiopathic medial necrosis, and its significance is still questionable.

The greatest age incidence of idiopathic cystic necrosis of the aorta in all cases so far reported is in the sixth decade, and they ranged from 25 to 83 years of age. Of the twelve acceptable cases in the literature, including the three described in this publication, seven were in men and five in women. In six instances the individuals were known to be at rest when the fatal rupture occurred, and only two were engaged in physical exertion at the time of the fatal seizure. The exact circumstances of the rupture in the other cases was either not known or believed not to be related to any unusual exertion. The initial seizure was known to be accompanied by pain in three instances, substernal in two and upper abdominal in one. The survival following the seizure varied, but in most instances death oc-

curred within a few hours. The longest survival was five days. In all cases the direct cause of death was cardiac tamponade, due to hemopericardium.

In all cases so far reported there has been cardiac hypertrophy, and in three of these, valvular heart disease. In five cases there was chronic renal disease in three of which there was a known clinical hypertension.

Sclerosis of large and medium-sized arteries was a common finding, but not out of proportion to the age of the individual. Diffuse vascular disease with arteriolar changes was seen in at least three cases and possibly more in which the descriptions were not explicit in this regard.

In the cases presented in this report, medial necrosis was most severe in the region of the rupture, but was not limited to that site. Tearing of elastic fibers and cystic degeneration was seen in the abdominal aorta of two of them.

The site of rupture in all of the cases so far reported was in the ascending arch just above the semilunar valves, and the direction of the tear was characteristically transverse. This is in accord with the observations on cases of spontaneous aortic rupture from any cause; and von Schnurbein [8] in a review of ninety-one cases found that in fifty-six of these the rupture was at this site, and that of the ninety-one cases the tear was transverse eighty-two times. The reason for this particular location of spontaneous rupture, as given by Oppenheim,[10] is that the ascending thoracic aorta has the greatest circumference and that since bursting tension is in direct proportion to the radius of the vessel, the smaller vessels can withstand higher pressure.

In this connection an investigation of the resistance of normal arteries of rabbits to intravascular pressure was made. Six young adult rabbits were used and each was given 20 mg. of heparin per kilogram body weight before the experiment to prevent clotting of blood. The aorta was cut just above the valve under ether anesthesia. The proximal end was ligated and into the distal end a cannula was tied and connected with a pressure bottle containing an india ink-saline injection mixture. The pressure within the injection system was controlled by means of a mercury manometer, which was connected directly with the cannula. The injection of fluid under high pressure was made into the living animal as rapidly as

possible, so that within two or three minutes after cutting the aorta intra-aortic pressures of 800 mm. of mercury or more were obtained. It was recognized that such pressures were not maintained at any considerable distance from the cannula, because of their rapid dissipation by flow of fluid into the veins. Loss of blood from the operative wound was prevented by overlapping Kelley clamps.

In all six rabbits, a sudden fall in pressure was noted after a pressure ranging between 800 and 1200 mm. of mercury had been obtained. This fall in pressure was followed by abdominal distention and rapid exhaustion of the injection mixture. The abdomen was opened, and by immersing the rabbit in running water the point of vascular rupture could be identified. In all six rabbits the rupture was either in the portal vein or in one of its primary tributaries. It was not possible in these experiments to rupture the arteries because of the rapid transfer of sufficiently high pressures to the veins to rupture them.

Macroscopic changes deemed characteristic were described by Erdheim and consisted of transverse, parallel-disposed, gray scars, marking the site of medial damage, beneath a thin, non-altered intima. Cyst formation within the media also reached macroscopic proportions.

The pathogenesis of the lesions resulting in the rupture appears to be quite definite so far as morphology is concerned. The etiology remains obscure, and we agree with Erdheim that it will be necessary to study many such cases to appreciate the conditions under which this peculiar type of necrosis develops. Erdheim has compared and distinguished these lesions from the medial changes seen in postinfectious cases by Wiesel,[21] from the postinfluenzal vascular damage described by Stoerk and Epstein,[22] and rheumatic arteritis by Pappenheimer and VonGlahn.[23] He has raised the question of avitaminosis or adrenalin poisoning as possible etiological agents.

This study suggests the probability that the disease is involutional or senescent in character. The damage results from the excess accumulation of chromatropic substance or mucin-like material, described by Schultz as representing an aging phenomenon in the arteries of animals and of men. Small cystic defects of the media were present in slightly less than 10 per cent of the sclerotic aortas of adults examined and were not necessarily identified with overlying atheromatous change (Fig. 5). The actual percentage may be

much greater since these were found in the course of a study of routine sections. It is true that the cysts were small, rarely involving more than three or four contiguous lamellae, and healing of such defects was not seen, but the differences between these defects and the lesions found in cases of spontaneous rupture were quantitative rather than qualitative. Without subscribing to Thoma's opinion as to the relation of medial weakening to atherosclerosis, it seems possible that the medial necrosis described in sclerotic aortas without rupture, as well as in the vicinity of spontaneous tears, is in accord with Thoma's idea of angiomalacia. This does not detract from the suitability of Erdheim's term "Medionecrosis aortae idiopathica cystica," but does throw a different light upon its significance.

Summary and Conclusions

Three cases of spontaneous rupture of aorta with cystic necrosis of the media have been described. The necrosis developed focally in areas the seat of chromatropic or mucinous degeneration, and was not associated with significant intimal change or inflammatory reaction. These have been compared with the previously reported cases of this disease and certain additional observations made. Tearing of the elastic elements occurred with and without cystic change and neither the cystic change nor the elastic tears were limited to the ascending arch of the aorta. The lesions were qualitatively similar to those commonly seen in sclerotic aortas, and differed in that the necrotic foci were larger and exhibited a greater tendency to repair. In the cases reported to date evidence of hypertension has been common but not constant, and in a considerable number the rupture has occurred while the individuals were at rest. Additional case studies will be profitable in establishing the similarity or dissimilarity of this disease to the changes commonly seen in arteriosclerosis without advanced intimal lesions.

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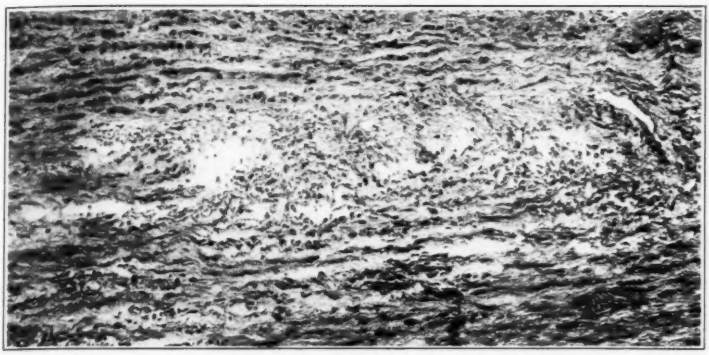
DESCRIPTION OF PLATES

PLATE 115

FIG. 1. (*a*) Hematoxylin and eosin and (*b*) orcein stains of same area in outer-third of media in two successive sections of same block from aorta of Case 1. An area of medial degeneration appears almost entirely healed in (*a*), but the extent of the elastic damage filled in by fibroblasts which do not conform to the lamellar structure of the media is seen in (*b*). Secondary degeneration has taken place in the repair tissue. × 124.

FIG. 2. Midportion of media of aorta from Case 2 near rupture. Chromatropic degeneration with cyst formation is seen in upper portion of photograph, while in lower portion the defect in the elastica is bridged by hyperplastic smooth muscle cells, the long axis of which is parallel to the elastic fibrils. The block was taken near the tear and extravasated erythrocytes are seen in the small cyst. Stained by orcein, according to Saphir. × 124.

FIG. 3. Midportion of media of aorta from Case 3. Stained by Sudan III to show fat droplets in the chromatropic substance between elastic fibrils and muscle cells near rupture. Wratten (C) filter. × 320.



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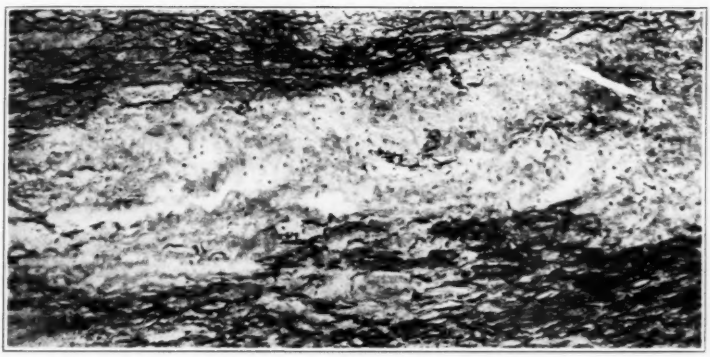

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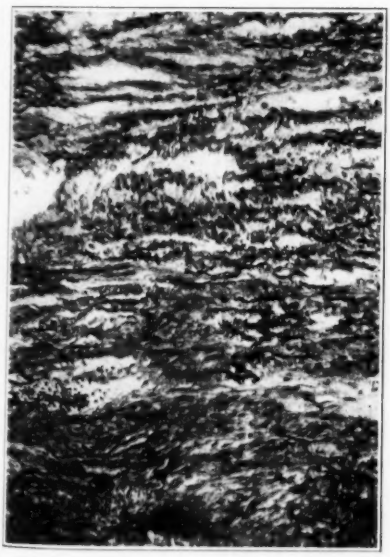


2

3

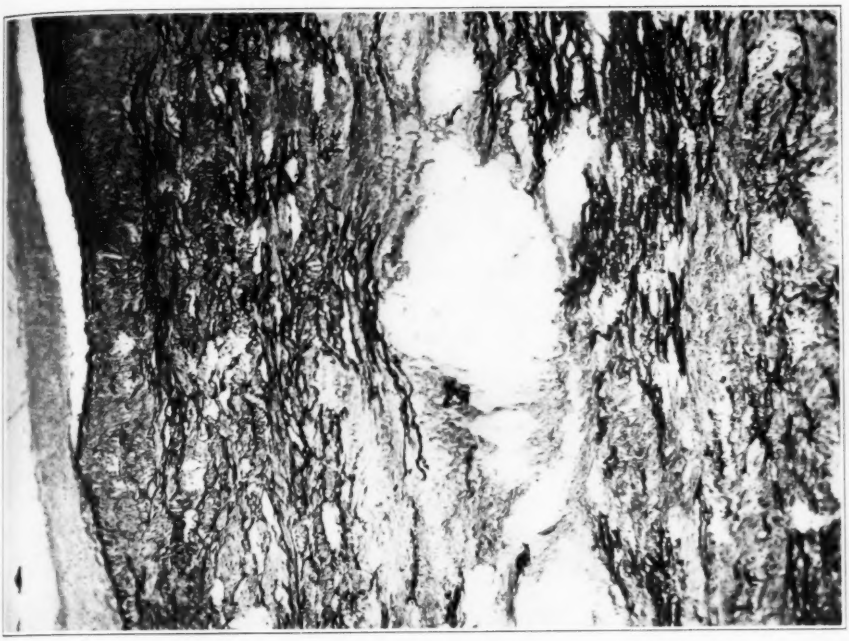
Moritz

Medionecrosis Aortae Idiopathica Cystica

PLATE 116

FIG. 4. The entire thickness of intima and media of aorta from Case 3, taken near rupture and including large cyst in midportion of media. Disruption of elastic fibrils is seen in the outer third with proliferation of fibrous connective tissue to repair defect. The intima is free from sclerosis, the internal elastic lamella is intact and there are no exudative cells. Stained by combination of the Verhoeff elastic and the Masson trichrome light green methods. × 75.

FIG. 5. (*a*) Hematoxylin and eosin and (*b*) orcein stains of same section of aorta of a 34 year old man who died of pneumonia. No syphilis and minimal intimal sclerosis. Focal increase of chromatropic substance with confluence of lamellae due to disruption of elastic fibrils. Probable precursor of more severe changes seen in "Medionecrosis aortae idiopathica cystica." × 134.



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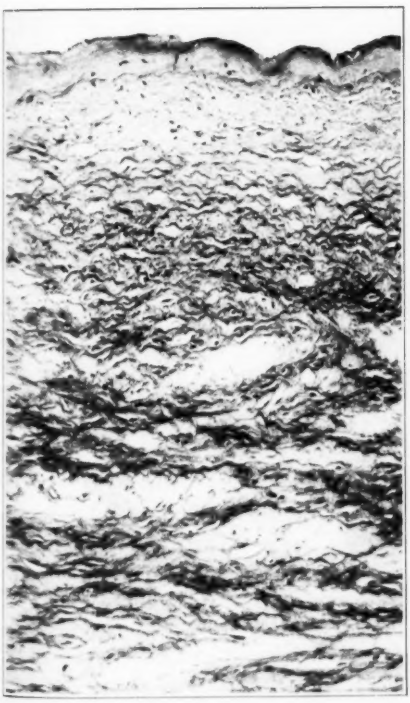

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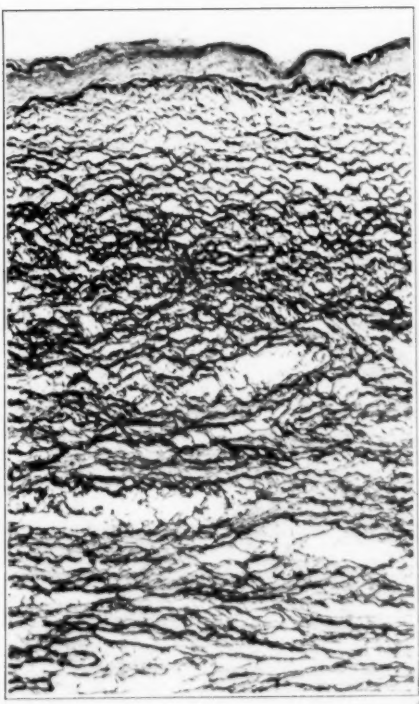

5 (*b*)

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Mediionecrosis Aortae Idiopathica Cystica

MESENTERIUM COMMUNE WITH INTESTINAL OBSTRUCTION *

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Anomalous secondary mesenteric attachments of both small and large intestine are common and present a wide variation in degree and location. An understanding of their pathogenesis rests upon a knowledge of fetal intestinal rotation. The description and definition of the successive stages of intestinal rotation and mesenteric attachment by Frazer and Robbins [1] is generally accepted and anomalies are classified according to that stage of development in which a departure from the normal occurred. A critical period in the development of the intestine is in or about the tenth week of fetal life when the gut returns to the abdominal cavity from the umbilical cord. Normally this return is accomplished in such a manner that the proximal portion of the colon lies in a plane ventral to the small intestine, crossing in front of the terminal portion of the duodenum from right to left. Subsequent rotation is counter clockwise and is followed by the secondary mesenteric attachments characteristic of the normal adult type.

The commoner anomalies of secondary mesenteric fixation are concerned chiefly with incomplete fixation of the ileocolic segment and have been adequately reviewed by Waugh.[2] One of the more extreme types of variation has been termed "mesenterium commune," which implies an absence of the secondary attachments to such a degree that the original prefixational type of suspension persists, that is, a common mid-dorsally attached mesentery for small and large intestine. The term "mesenterium commune," however,

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Dr. W. E. Ladd has described three cases of mesenterium commune with volvulus and intestinal obstruction in a published study (Ladd, W. E. Congenital obstruction of the duodenum in children. *New England J. Med.*, 1932, **206**, 277), and has been so kind as to permit the inclusion in this report of a fourth case which has not been published (personal communication). These were cases of clockwise volvulus of the entire jejunum, ileum and proximal half of the colon in children who were operated upon at 2 weeks, 4½ weeks, 9 months and 1 year of age respectively, for obstruction of the third portion of the duodenum. All four children survived the operation with subsequent relief from obstruction. Because so much of the intestine was included in the volvulus Ladd has stressed the necessity of "delivering" the whole small bowel and untwisting it.

has not been restricted arbitrarily to conditions so completely primitive, but has been applied to the suspension of the small intestine from a midline pedicle affording great mobility to the small intestine and proximal portion of the colon (Anders [3]).

Anders, in a comprehensive review of congenital malformations of the intestine and mesentery, states that a "mesenterium commune" exists in all cases in which there has been complete or advanced retardation of intestinal rotation. The correctness of his conclusion seems apparent, since proper fixation would depend upon proper position, but it does not follow that proper rotation would always predicate normal secondary attachments. It seems likely that regardless of the manner in which the intestine rotates in its return to the abdominal cavity the hypermobility permitted by a "mesenterium commune" may lead to a wide variation in the position observed in postfetal life. Dott,[4] and Haymond and Dragstedt [5] have reviewed the various types of abnormal position and have considered them varying degrees of reversed rotation, rather than of incomplete rotation.

A consideration of the association of "mesenterium commune" with incomplete rotation in a normal direction, as well as with reversed rotation, is of more than academic interest. Many of the cases presented by Dott and by Haymond and Dragstedt are characterized by intestinal obstruction due to volvulus. If the "mesenterium commune" existing in such a case were due to a primary reversed rotation which occurred *in utero*, the obstructing volvulus would have to be corrected in such a manner as to bring the intestine back to its original position of reversed rotation, because a position acquired by the intestine in the tenth week of fetal life would have become, in all probability, normal for that individual.

Recently four cases of "mesenterium commune" have come under our observation, two of which have simulated reversed rotation because of volvulus.

Case Reports

Case 1. Mesenterium Commune

Clinical History: A white, female, cretin child, 7 months of age, was admitted to the Babies' and Children's Hospital and died following a respiratory infection without any significant gastro-intestinal complaints.

Postmortem Examination: In addition to hypoplasia of the thyroid gland and an acute bronchitis there were defective secondary mesenteric attachments.

On opening the abdomen the cecum was found to be in the epigastrium in the midline, with the colon occupying the left and the small intestine the right side. The greater omentum was undeveloped. The entire jejunum and ileum were suspended from a common mesenteric axis in the midline. The cecum and proximal portion of the colon were suspended by a redundant mesentery which extended upward from the pedicle of attachment of the small intestine and then to the left with descent on the left posterior surface of the abdominal wall toward the pelvis. The configuration of the posterior mesenteric attachment was that of an interrogation point (?), (Text-Fig. 1). The superior mesenteric artery crossed over the third portion of the duodenum in the usual fashion. Because of the hypermobility permitted by the defective attachments it was possible to rotate the small intestine, cecum and proximal portion of the colon through an arc of 360° in a clockwise direction and bring the colon behind the duodenojejunal junction, and by reason of the torsion of the proximal portion of the jejunum the superior mesenteric artery assumed a position between the small and large intestine. Figures 1, 2 and 3 show the successive stages of this manipulation and Figures 4, 5 and 6 are diagrams indicating the relative changes in the relation of small and large intestine and superior mesenteric artery (see legends).

Case 2. Mesenterium Commune with Intussusception

Clinical History: A white, male child, 9 months of age, was admitted to the Babies' and Children's Hospital because of abdominal pain, vomiting and bloody stools of three days duration. The onset of the illness was characterized by vomiting, followed by intermittent paroxysms of pain. The past and family history were negative.

The child was in constant distress with frequent acute attacks of pain. The abdomen was tense, rounded and rigid. There was a large fusiform mass palpable in the left side and extending into the rectum. Peristalsis was active. Laboratory examination was not significant, other than a count of 16,400 white blood cells.

The patient was transferred to Lakeside Hospital for operation by Dr. J. W. Holloway. At operation the cecum was found to be situated to the left of the midline, high in the abdomen, and there was an intussusception of a large part of the small intestine into the colon, the tip of the intussuscepted bowel being palpable in the rectum. The intussusception was completely reduced by a com-

bination of traction above and massage below. The intestine appeared viable and in surprisingly good condition, save for edema of the cecum and ascending colon. Silk sutures were employed to fix several inches of the ileum to the wall of the cecum to prevent a recurrence of the intussusception. After reduction of the obstruction the large intestine occupied the left side of the abdomen and the small intestine the right. The large intestine was suspended on a long mesentery and was unusually mobile.

The child died twenty hours after operation with a clinical diagnosis of acute peritonitis.

Postmortem Examination: Twenty centimeters of the lower ileum were hemorrhagic, dilated and edematous. The mucosa was necrotic and the lumen contained sanguineous fluid. There were several silk sutures attaching the ileum to the wall of the cecum. There were 50 cc. of serosanguineous fluid in the abdominal cavity.

As in the preceding case the secondary mesenteric attachments were defective. The base of the mesentery on the posterior abdominal wall followed the general outline of an interrogation point (?), the upper limb starting just below the superior mesenteric artery in the midline from which the line of attachment ascended slightly, then extended to the left side of the abdomen and down to the pelvis in the midline. The mesentery was long, permitting great mobility of both small and large intestine and they could easily be manipulated by rotating the small intestine clockwise on its mesenteric pedicle until the superior mesenteric artery was brought to a position behind the duodenojejunal junction and the colon into a plane posterior to the small intestine. The possibility of this rotation was significant in the understanding of the succeeding two cases.

There were no other significant pathological changes.

Case 3. Mesenterium Commune with Volvulus

Clinical History: A white, male infant, 3 weeks of age, was admitted to the Babies' and Children's Hospital with a history of persistent vomiting. The family history was not significant. There had been no bowel movements from birth although a small amount of green material was passed after enema. The child had taken water and feedings eagerly but had vomited after each feeding. Roentgenological examination after barium by mouth indicated obstruction to the third portion of the duodenum which was not relieved by atropin. The duodenum proximal to the obstruction was dilated and the barium gradually passed the point of obstruction.

The patient was transferred to Lakeside Hospital and operated upon by Dr. J. W. Holloway. The stomach and duodenum were greatly distended and there was a palpable cord-like obstruction in the third portion of the duodenum.

The cecum was adherent to the anterior surface of the duodenojejunal junction and upon freeing it the colon was seen to pass from right to left behind the twisted segment of duodenum. The colon was fixed in this position by adhesion to the posterior abdominal wall. After the cecum had been dissected free it was possible to demonstrate the patency of the obstructed portion of the duodenum by pressing on the stomach. The operative note is concluded with the statement that "however, one could not establish normal relations by any process of rotation. In view of the fact that the duodenum at least was anatomically patent, it was felt that nothing could be accomplished by further exploration."

The child died on the second day after operation.

Postmortem Examination: Three distinct abnormalities were disclosed by examination of the abdominal contents.

The first anomaly was defective mesenteric attachment, similar to that described in the previous cases in which the distal portion of the duodenum was unusually mobile, the small intestine suspended from a common mesenteric root, the axis of which was the superior mesenteric artery, and the colon suspended on a long mesentery whose posterior attachment followed a line which curved to the left and then descended toward the midline of the pelvis.

The second anomaly consisted of a clockwise volvulus of 360° whereby the duodenum had been drawn anterior to the superior mesenteric artery and the colon posterior to it, with fixation of the colon by fibrous adhesions in that position (see Fig. 4). The twisted segment of duodenum was surrounded by fibrous adhesions but was not completely obstructed. Most of the adhesions had been dissected free at the time of operation, and there was considerable hyperemia and edema at this site. Both on gross and microscopic examination the absence of injury to the muscularis indicated that the twisted segment of the gut had not been severely damaged and that the volvulus had not caused any considerable degree of stenosis.

The third anomaly was an intra-abdominal hernia by which a large part of the small intestine had passed through a defect in the mesentery close to the ileocecal junction. The aperture in the mesentery was about 1 cm. in diameter and the margins of the orifice were slightly thickened. This mesenteric hernia was apparently the condition responsible for the surgical failure to re-establish normal relationship. It was first necessary to reduce the hernia, which was done without difficulty and without dissection. The colon was freed of its secondary adhesion behind the duodenum and the large and small intestine rotated in a counter clockwise direction through an arc of 360°. This returned the large and small intestine to their

former places, although the condition was still that of a left-sided colon. The duodenum was now behind the superior mesenteric artery.

Case 4. Mesenterium Commune with Volvulus

Clinical History: The patient was a white, male infant, 7 weeks of age, who entered the Babies' and Children's Hospital because of persistent vomiting. Despite the vomiting after each feeding there had been a gain of 1½ pounds since birth and occasional well formed stools had been passed. Roentgenological studies showed the stomach and duodenum to be dilated, with partial obstruction at the duodenojejunal junction. Although most of the barium was vomited, some passed through the large intestine in eighteen hours.

The patient was transferred to Lakeside Hospital and operated upon by Dr. F. S. Gibson. The terminal portion of the duodenum was obstructed by a volvulus of the entire jejunum, ileum and part of the colon. The colon was posterior to the duodenum and so firmly fixed by adhesion that anatomical restitution was not considered possible.

The child died four hours after operation.

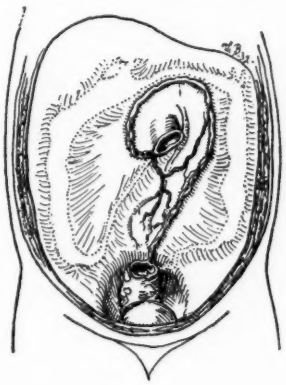
Postmortem Examination: The intestinal malposition was essentially that described in the operative note. The mesenteric attachments of the colon were entirely to the left of the midline. The distal portion of the duodenum was mobile, and it, as well as the remainder of the small intestine, was attached by means of a narrow pedicle in the midline which took origin below the region of the superior mesenteric artery and extended inferiorly and to the right of it for a short distance. There had been a twist of 360° in a clockwise direction of the entire jejunum and ileum and proximal colon, so as to bring the colon behind the duodenojejunal junction, where it was fixed by dense fibrous adhesions (see Fig. 4). The superior mesenteric artery was also brought to a position between the duodenum and colon. After freeing the fibrous adhesions the colon and artery were returned to their proper place in relation to the small intestine by rotation through 360° in a counter clockwise direction. The duodenal mucosa at the site of the twist was edematous and hemorrhagic and the muscularis was fibrous. There was no change, other than the serosal thickening, in the wall of the colon where it was fixed behind the duodenum.

Discussion

Four cases presenting defective secondary mesenteric attachments of the intestine of the type commonly called "mesenterium commune" are described. The attachment of the mesentery to the

posterior abdominal wall followed a pattern similar to an interrogation point (?), the small intestine being suspended from a pedicle, beginning at the upper limb of the ? with the line of attachment of the colon, deviating to the left and then descending toward the midline into the pelvis (Text-Fig. 1). In none of the cases was the mesentery of the colon fused with the greater omentum and in all the greater omentum was vestigial.

The first case was of an infant, 7 months of age, in whom the defective mesenteric attachments were an incidental autopsy finding without any clinical record of obstructive phenomena.



TEXT-FIG. 1

Drawing illustrating the dorsal line of mesenteric attachment as seen in all four cases (solid lines) in contrast to the normal secondary line of dorsal attachment that should have been present (dotted lines).

In the second case, which was of an infant, 9 months of age, the defective mesenteric attachments had made possible an intussusception of the small into the large intestine of such magnitude that the advancing end of the intussuscepted gut could be palpated by a rectal examination.

In Case 3 and Case 4, which were of infants, 3 and 7 weeks of age respectively, the condition disclosed at operation and autopsy simulated reversed rotation in the second stage of intestinal development (Frazer and Robbins). The colon was fixed behind the small intestine at the duodenojejunal junction and the superior mesenteric

artery lay between the small and large intestine (Fig. 4). That this malposition was a volvulus rather than reversed rotation during fetal life, was indicated by the torsion of gut and mesentery at the axis of rotation which was at the duodenojejunal junction. The abnormal mobility of the terminal portion of the duodenum prevented the superior mesenteric artery from being obstructed by being included in the twisted segment. The manipulation required for the relief of the obstruction brought the colon and the superior mesenteric artery anterior to the duodenojejunal junction and in their normal planes. Figure 4 indicates the abnormal relations incident to the volvulus and Figures 5 and 6 show the successive stages in the reduction of the volvulus by a counter clockwise rotation of 360° as indicated by the arrows. The relations obtained by this corrective manipulation (Fig. 6) represent those of normal fetal rotation but with the defective secondary fixation illustrated in Text-Fig. 1.

Inasmuch as such conditions are likely to be seen first with a limited surgical exposure it is important to appreciate how closely a volvulus resulting from a "mesenterium commune" may simulate abnormal fetal intestinal rotation. The manipulation required for the relief of a volvulus due to defective mesenteric fixation in an intestine which has rotated normally is obviously quite different from that which would be effective in relieving an obstruction in a malposition of the intestine because of abnormal fetal intestinal rotation. In either instance the intestine would have to be returned to the position normal for that individual, whether that position be the result of the usual or of reversed direction of fetal rotation. Regardless of the direction of fetal rotation the position of the intestine resulting from it would have become normal for the individual, inasmuch as the direction of rotation is established by the end of the tenth week of fetal life and all further growth of intestine and mesentery are conditioned by it.

Conclusions

Four cases of "mesenterium commune" have been described, one in an infant manifesting no disturbance referable to the anomaly, and three in infants who died of intestinal obstruction secondary to the hypermotility engendered by the defective mesenteric attach-

ments. In two of the infants, the obstruction was caused by a volvulus of 360° in a clockwise direction, which because of the abnormal motility of the terminal portion of duodenum reversed not only the planes of the small and large intestine, but also the relation of superior mesenteric artery to the duodenojejunal segment of the intestine. The condition in these two cases was such as to stimulate reversed developmental rotation.

These cases illustrate the surgical necessity of determining at the outset whether the condition is a real reversed rotation or a secondary volvulus due to defective fixation. Correction of a true reversed rotation established during the third month of fetal life must regard the reversal as normal for that individual, while only the volvulus type may be reduced by returning to normal position.

The author wishes to thank Dr. J. W. Holloway, Dr. F. S. Gibson, and the Babies' and Children's Hospital for the use of their records, Miss Theodora Bergsland for the drawings and Dr. B. M. Patten for helpful advice.

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DESCRIPTION OF PLATE

PLATE 117

FIGS. 1, 2 and 3. Photographs of the successive stages in the reduction of the volvulus seen in Cases 3 and 4. (See Figs. 4, 5 and 6 for interpretation.)

1. Clockwise volvulus of 360° with reversal of the planes of large and small intestine.

2. Reduction of the volvulus by 180° in a counter clockwise direction.

3. Complete reduction of the volvulus with establishment of normal position.

FIGS. 4, 5 and 6. Drawings showing the relations of small and large intestine to one another and to the superior mesenteric artery in the photographs shown in Figs. 1, 2 and 3.

4. Volvulus of 360° as shown in Fig. 1. The arrow indicates the direction of rotation necessary for reduction of the volvulus.

5. Reduction of the volvulus by 180° as shown in Fig. 2. The arrow indicates the direction of rotation for complete reduction.

6. Complete reduction with establishment of normal position.

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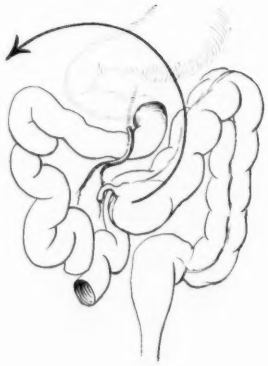

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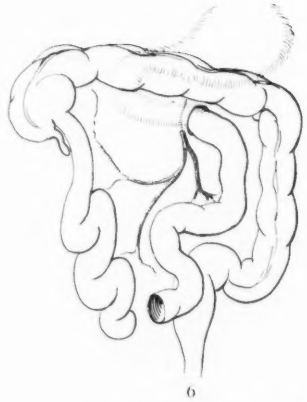

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Moritz

Mesenterium Commune with Intestinal Obstruction

FIBROCYSTIC DISEASE OF THE BONES ASSOCIATED WITH TUMOR OF A PARATHYROID GLAND *

REPORT OF A CASE

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Generalized fibrocystic disease of bone was first described as generalized osteitis fibrosa cystica by von Recklinghausen[1] in a Festschrift to Virchow in 1891. The earliest references we have to tumor of the parathyroid glands are those of De Santi[2] in 1900 and Benjamins[3] published in 1902; bone disease was not mentioned. Erdheim[4] in 1903, Hulst[5] in 1904, and MacCallum[6] in 1905, also each reported a case without associated bone disease. Askanazy[7] reported finding a parathyroid tumor in association with osteitis deformans in 1904, and von Verebély[8] reported a case of parathyroid tumor with bone changes in 1907. Weichselbaum[9] described a parathyroid tumor in 1906 without associated bone changes. Erdheim[10] described three cases of osteomalacia with parathyroid enlargement in 1907. Thompson and Harris[11] described a similar case in 1908. Seven cases of parathyroid tumor without mention of bone changes were collected and one of his own added by Da Costa[12] in 1909. Bauer[13] in 1911 reported a case of adenoma of the parathyroid in a 45 year old woman with a moderate degree of osteomalacia. In 1913 Molineus[14] described osteomalacia in three elderly females, two of whom had each one parathyroid tumor, while the third had two distinct tumors of parathyroid tissue. Harbitz[15] in 1915 noted the "relationship between enlargement of the parathyroid glands, rickets and other diseases affecting the bones."

Tumors of the parathyroid gland have been reported as coincident findings in several diseases. Bergstrand[16] in 1921 reported tumor of the glands in nephritis, tetany, epilepsy, eclampsia and osteomalacia.

* This case was reported in abstract before the Buffalo Pathological Society on February 19, 1932.

Received for publication April 5, 1932.

In 50 cases of nephritis he found one or more enlarged parathyroid glands. Other authors, including MacCallum, have reported the coincidence of parathyroid enlargement with nephritis. Hoffheinz [17] in 1925 collected 45 cases of parathyroid tumors; in 17 of these there was an associated generalized osteitis fibrosa cystica, 8 cases with osteomalacia, and 27 with various other bone diseases.

The concept of the relation between tumors of the parathyroid gland and generalized fibrocystic disease has been changing within the last few years.

Erdheim in 1911 [18] put forth the hypothesis that the enlargement of the gland was a compensatory effort to assist in replacing lost calcium to the bones. This theory held sway for many years.

In 1923 Dawson and Struthers [19] stated that the glandular hyperplasia was an effort to prevent the excessive excretion of lime salts and also control and prevent the development of an excess amount of guanidine, which was considered toxic. The first intimation that hyperparathyroid function might be a cause of generalized fibrocystic disease of bones through withdrawal of calcium was in 1915, when Schlagenhaufer [20] advised parathyroidectomy in two cases of generalized fibrocystic disease associated with a parathyroid tumor: Maresch favored the procedure but Bauer rejected the proposal. In 1925 Mandl [21] transplanted parathyroid tissue into a patient with generalized fibrocystic disease and the patient's condition became worse. He then removed the transplanted parathyroid tissue and a parathyroid tumor, and there was improvement of the fibrocystic disease. Gold [22] performed a parathyroidectomy in 1927, with similar improvement of the patient's bone disease.

Barr and his coworkers [23] had a parallel case in 1929. Since this time there have been reported 23 cases of generalized fibrocystic disease of bones associated with parathyroid tumor, in each of which the tumor was removed, with subsequent improvement of the bone condition.

Collip in 1925 [24] described blood calcium elevation after parathormone was injected in dogs, and also an increase in blood phosphorus in parathyroidectomized rabbits.

Greenwald and Gross in 1925,[25] and again in 1926,[26] showed that daily parathormone injection of 100 units in animals elevated the serum calcium and caused excretion of urine calcium and phosphorus, the former to as much as six times normal. Hunter and Aub [27] also

found the same hypercalcemia with increased calcium excretion in man in 1926.

Albright, Bauer, Ropes and Aub[28] have demonstrated a negative calcium balance in animals and man receiving parathormone injections. Finally, the disease picture, both gross and microscopic, of generalized fibrocystic disease of bones has been produced repeatedly by Jaffe, Bodansky and Blair,[29] and also by Byrom working with Hunter and Turnbull.[30] More recently Johnson and Wilder[31] reported that repeated injections of parathyroid extract produced in puppies and young rats uniform bone lesions characteristic of generalized fibrocystic disease of the bones, and concluded that the disease observed in man was due to an oversupply of parathyroid hormone with consequent loss of bone calcium.

Thus the interrelationship of generalized fibrocystic disease of bone and hyperparathyroidism has gradually been established. We have found 31 reported cases of generalized fibrocystic disease of bones associated with enlargement of a parathyroid gland. Some of these were reported as hyperplasia, others as adenomas, and one as a malignant adenoma. All of these present definite clinical and laboratory data to establish further the causal relationship of these two conditions. One additional case is presented herewith.

Case Report

Clinical History: D. S., age 50 years, white, female, married housewife, entered the Buffalo City Hospital April 9, 1931, complaining of muscle aches, fatigability, pain about the knee joints and lower part of the right tibia, and loss of use of the lower limbs because of weakness.

As a young girl she had had a goitrous swelling of the neck for which she was treated with iodine. Following this the swelling disappeared. At the age of 27 years she had a periapical tooth infection that left her with a condition that was termed chronic osteomyelitis; this resisted all ordinary attention, a sinus persisting until a radical surgical procedure caused it to heal after two years.

Constipation had been obstinate for thirteen years. Seven years ago she had what was interpreted as sciatica, the pain being in both gluteal regions. For the past three years a sense of soreness was present in both heels; this was aggravated by weight-bearing. She had lost 71 lbs. in weight in this three year period, the former maximum weight being 160 lbs. About three years prior to hospital admission she had had an attack of sharp, right upper quadrant pain with sudden onset and cessation, which was interpreted at the time as renal colic.

For the last two years needle-like pain was experienced around the knees; for the past five months a pain was sensed over the lower third of the right tibia. This, aggravated by motion, has been present constantly.

She had been conscious of a firm swelling in the right thyroid region, and more recently a sense of tightness. There had been no dysphagia. Nervousness, force-

ful pulsation of the neck vessels, and a fine tremor had been present for three years. A precordial pain had been interpreted at one time as pericarditis. Night and day frequency of urination was constant.

The patient was an elderly, emaciated female with drawn and haggard features. The bony prominences and hollows were marked. The skull was seemingly larger than normal in contrast with the face bones. There was a slight exophthalmos and a suggestion of nystagmus; the sclerae had an icteric tinge. The neck vessels pulsated forcibly; a tender firm nodule was palpable in the lower lateral portion of the right thyroid lobe. Most of the teeth were missing; the few remaining ones were carious. The heart was slightly enlarged to percussion, the left border measuring 8.5 cm. to left of the midline in the fifth intercostal space, and the right 3.5 cm. to the right of the midline in the fourth interspace; there were two cardiac murmurs, a mitral systolic blow and a rough aortic systolic. Extra systoles were frequent. The systolic blood pressure was 122, and the diastolic 68. No arteriosclerosis was detected. The muscles of the extremities were atrophic, their tone poor. A slightly tender area was found over the lower third of the right tibia. The calcaneal regions were tender.

Pulse and respirations were normal. The temperature occasionally rose to 99.8 and 100° F. All other points of the anamnesis were negative.

Laboratory Studies: Red blood cells 2,450,000; hemoglobin 58 per cent (Sahli); color index 1.2; white blood cells 10,100; differential normal. A series of blood calcium determinations yielded 16.5, 13.82, 14.52 mg. per 100 cc. The blood phosphorus was 2.3 mg. per 100 cc. of blood. Urine: specific gravity ranged constantly between 1006 and 1013; albumin was 1 plus to 4 plus; occasional rare granular cast. Bence-Jones protein reaction was positive on one occasion and negative on another. Basal metabolic rate was within normal limits. The X-ray revealed cystic areas in the ribs, clavicle, scapulae, humerii, tibiae and mandible, vacuolization of the skull and a generalized loss of density of all the bones.

Biopsy material from the right tibia was obtained April 18, and was diagnosed as fibrocystic disease of bone.

On May 15, 1931, a tumor mass was removed from behind the right thyroid lobe. Local anesthesia was used. Following this the patient became stuporous with nervousness, apprehension, clonic twitchings of hand and face muscles, and could be aroused only by parathyroid and calcium therapy. The serum calcium determination on the same day, after 20 cc. of 5 per cent calcium chloride and 2 cc. parathormone was injected intravenously, was found to be 9.2 mg. per 100 cc. of blood.

With the onset of dyspnea, cyanosis and vasomotor collapse, an acute urinary suppression supervened. A blood urea determination showed 68.5 mg. per 100 cc. of blood. Death followed four days after operation. Autopsy was refused.

Bone Biopsy

The material received from biopsy* consisted of two fragments of bone each 1 cm. square and 3 mm. thick, and two bone fragments about 2 mm. in diameter, one minute piece of soft white tissue about 1 mm. in diameter, and some blood clot bulking about 1 cc.

* The bone biopsy and the parathyroid gland tumor were submitted by Dr. H. N. Kenwell and Dr. Pietro Blanco of the Buffalo City Hospital.

The sections stained with hematoxylin and eosin exhibited strands of osteoblastic tissue running at irregular angles from the periphery inward, forming a totally irregular pattern with no osseous structures present. The cells of the strands had a very scanty, faintly staining cytoplasm; their nuclei tended to be round or oval, and had within them a deeply staining chromatin material within which darker granules were seen with the aid of the oil immersion objective. Some thin-walled vascular structures were found throughout the section. The above type of cells yielded gradually by transition to larger paler cells with paler staining nuclei, the cytoplasm being more abundant and of a granular nature. Adjacent to and between these latter cells a mature fibrous reticulum was noted in a background of homogeneously light grayish pink vacuolation. Blood vessels in these latter areas were scarce, but here and there in the large vacuoles an isolated red blood cell was seen.

In this and other sections multinucleated large cells were found scattered throughout the fields, but chiefly in and around the vascular, younger and more active appearing osteoblastic areas. These cells approximated 15 to 20 microns in diameter. The cytoplasm was stained a soft grayish pink and the nuclei pinkish blue; these were round and oval and situated eccentrically, and had sharp outlines. They contained mostly a nucleolus and in some cases many small dark blue granules. The nuclei approximated about 4 microns in diameter. The greatest number of nuclei counted in any one giant cell was 28; the least number was 5. The cytoplasm of the multinucleated cells was irregularly and faintly outlined and within the cytoplasm of several of them fragments of red blood cells were seen.

In another section of bone the osseous tissue had been partly replaced by osteoid tissue stained a light pink, and in some places a deeper bluish pink. The homogeneity of the osteoid tissue was broken only by irregularly situated elliptical cells in lacunae. These cells had scant cytoplasm and oval nuclei with poorly staining chromatin material.

Between the osteoid areas, and dipping into them in finger-like processes were groups of osteoblasts and fibrous tissue strands. At the periphery of the osteoid tissue the osteoblasts were larger than elsewhere, the cytoplasm was clear and the nuclei were stained a deep blue. The cells close to the areas of osteoid tissue were larger and had more deeply staining chromatin than those farther away.

As before, multinucleated cells or osteoclastomata were seen, but they were not so large as those previously described, and they had a deeply staining nucleus. They were concentrated here more on the inner (marrow) aspect of the osteoid tissue. Between the limiting membrane of the osteoclastoma and the surrounding structure there was in many instances a clear area. One received the impression that the osteoclastic activity here was about two or three times that of the osteoblastic process.

One cc. of a brownish red fluid was obtained from a cyst in the right tibia during the biopsy. Direct smears of this showed an occasional Gram-positive short bacillus, thought to be contamination. The Rivalta test was 4 plus. Cultures did not exhibit any bacterial growth. Smears of the fluid stained with hematoxylin and eosin showed many normal appearing red blood cells, about five lymphocytes per high power field, and a rare three or four-lobed polymorphonuclear neutrophilic leukocyte. In one field there was seen a faintly pinkish gray, vacuolated, roughly circular "ghost" cell, which contained darker bluish pink, indistinctly outlined structures. This looked as though it might be a degenerated osteoclast.

The tissue diagnosis was fibrocystic disease of bone.

Parathyroid Tumor

The nodule removed from the thyroid region was a piece of tissue that weighed 7 gm. This was irregularly elongated and somewhat V-shaped. It measured 2 cm. through its long diameter and 2.5 cm. across, and was dark brown in color. It appeared to be well encapsulated.

Sections of the tumor stained with hematoxylin and eosin exhibited a thin fibrous capsule from which bundles of connective tissue swept centripetally, septating the glandular parenchyma. A delicate fibrous reticulum supported the glandular cells. These resolved themselves into several types. There were lobules of brighter, pinker staining tissue which were found to be chiefly large polyhedral cells, with a moderate deeply staining granular cytoplasm and oval nuclei which were a light pinkish blue with few granules. The cells had an alveolar arrangement in some fields, and the alveolar spaces contained a pinker staining, amorphous material. Scattered among these pale oxyphilic cells were basophilic or principal cells.

These latter had slightly larger nuclei which stained a purplish blue and contained coarse granules. In one section basophilic cells formed alveoli containing an amorphous acidophilic substance. The principal or basophilic cells were scanty in number and the cell membrane was not clearly seen. Occasionally an acidophilic cell was seen among the nests of basophilic cells.

An iron stain, using the Prussian blue reaction, showed some small granules of black material in some of the connective tissue septae.

A fat stain (scharlach R) revealed some intercellular fat globules, mostly in a region of the chief cells, but also in some of the capillaries.

Small, thin-walled capillaries were frequently seen among groups of cells. There was one field that showed cystic change with a brownish staining pigment in the cystic areas and in the surrounding connective tissue structures. No mitoses were seen. Only two types of glandular cells were noted. No evidence of irregularity of the cellular proliferation was noted.

The tissue diagnosis was adenomatous hypertrophy of a parathyroid gland, with cystic degeneration.

Summary

1. The literature concerning the development of the concept of the relation of generalized fibrocystic disease of bones to hyperparathyroidism has been reviewed.

2. A case of generalized fibrocystic disease of bone in correlation with a tumor of parathyroid gland has been presented.

The author is indebted to Dr. William F. Jacobs, Pathologist-in-Chief of the Buffalo City Hospital for the use of this material, and for his criticism in its development.

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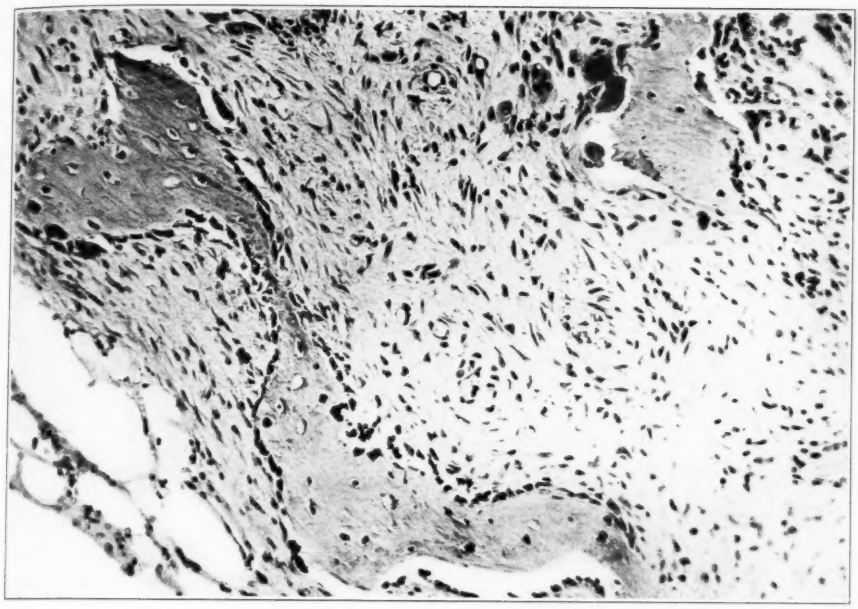
DESCRIPTION OF PLATE

PLATE 118

FIG. 1. Bone biopsy. Hematoxylin and eosin stain. Hyperchromatic osteoblasts are seen at the edge of one island of osteoid tissue. Osteoclastomata appear at the periphery of another osteoid island. There is a considerable degree of fibrous tissue between the osteoid structures.

FIG. 2. Parathyroid tumor.

FIG. 3. Parathyroid tumor. Hematoxylin and eosin stain.



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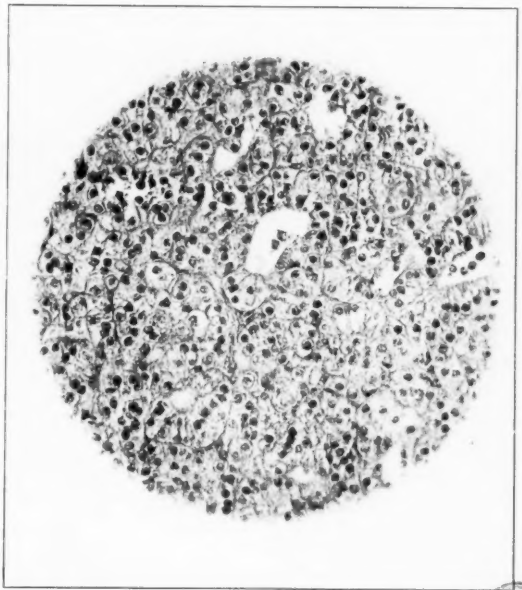

3

Rosedale Fibrocystic Disease of Bone

A STUDY OF THE PATHOGENICITY OF THE BACILLUS OF CALMETTE-GUÉRIN (B.C.G.) *

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In the use of a living microörganism as a means of establishing an immune state that will be of definite protective value against spontaneous infection of a virulent bacterium, it is important that the particular microörganism used for vaccination be devoid of the ability to produce lesions of a progressive character. According to Calmette and Plotz [1] the particular bacillus of tuberculosis, known popularly as B.C.G., is definitely and permanently innocuous, although it is recommended that to ensure the perpetuity of the avirulent state the organism should be grown on glycerine-broth-potato medium with a change at certain intervals to glycerinated ox bile-potato medium, or the liquid medium of Sauton.

The contention that the avirulence of B.C.G. is of a fixed character has provided an impetus for considerable experimental work in an attempt to determine the validity of Calmette's assertions. The reports of Watson,[2] Petroff,[3] Hutyra,[4] Kraus,[5, 6] Malkani,[7] and others would indicate that under certain conditions unquestionable evidence of pathogenicity of varying degrees may occasionally occur in experimentally exposed animals. On the other hand, the results of the investigations of Remlinger and Bailly,[8] Okell and Parish,[9] Gerlach,[10] Haring and his coworkers,[11] and Griffith [12] would indicate that Calmette's claim for the avirulence of B.C.G. is essentially correct.

The attempts to demonstrate the pathogenic propensities of the bacillus of Calmette-Guérin by the foregoing writers were done for the most part with cultures that were grown according to the procedure recommended by Calmette. Since, however, as pointed out by Petroff, all control of the environment of the organism is lost

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when it enters the animal body, a microörganism intended for vaccination should maintain a condition of avirulence regardless of various environmental influences to which it may be exposed. It therefore becomes exceedingly pertinent to know something of the biological behavior of B.C.G. after it is grown on culture media different from those approved by Calmette. In this regard the work of Sasano and Medlar [13] is of interest. Although these observers found that guinea pigs and rabbits could be inoculated with as much as 20 mg. of B.C.G. grown on bile-glycerine-potato medium without evidence of progressive tuberculosis, marked virulence was noted after their particular strain of the organism had been cultivated on a modified Sauton medium adjusted to pH 7.2 to 7.4 just prior to inoculation. Immediately before inoculation 10 per cent normal, unheated rabbit serum was added to the medium. The medium was seeded with a portion of a bile-glycerine-potato culture of B.C.G. which was procured in 1926 from Dr. W. H. Park of New York City and subcultures were made every four weeks. At the time of each subculture 2 rabbits were usually inoculated intravenously with 1 mg. of the culture. Beginning with the third subculture, 11 rabbits were inoculated to and including the eighth subculture, and marked tuberculous lesions were observed in each of the rabbits. Acid-fast bacilli were numerous in the tissue in all cases, with the disease most pronounced in the lungs. The spleen and kidneys were also affected, although lesions were not common in the liver. The infected rabbits died in from eleven to fifty days; most of them died in from twenty to thirty days after inoculation. Animal inoculations from Subcultures 1 and 2 were not mentioned. Pathogenicity of the subcultures grown on the modified medium of Sauton was also demonstrated for guinea pigs and for calves.

Sasano and Medlar wrote: "B.C.G., when grown under the environment which we succeeded in establishing, has evidenced a virulence greater than any other culture of tubercle bacillus we have had in our laboratory." They concluded that B.C.G. is not a fixed virus and that the stability of its state of virulence depends entirely on its environment.

The striking results obtained by Sasano and Medlar caused Boquet [14] of the Pasteur Institute to attempt to duplicate the experiment. The medium of Sauton was prepared according to the procedure followed by Sasano and Medlar, and three series of subcul-

tures were prepared using three different strains of B.C.G. At the time of making each subculture, guinea pigs and rabbits were inoculated, the former subcutaneously and intraperitoneally and the latter intravenously. Sixty-nine guinea pigs and 33 rabbits were inoculated with the respective subcultures, and although the dosage used was ten to fifteen times greater than was used by Sasano and Medlar, autopsy of the animals did not reveal the slightest evidence of tuberculosis. Having failed to confirm the observations of Sasano and Medlar, although the same technique was used, Boquet was of the opinion that although it is possible that some investigator in the future might succeed in modifying the pathogenicity of B.C.G., irrefutable proof of such modification has not as yet (1931) been recorded.

The remarkable divergence of the results obtained by what were presumably identical procedures makes it impossible to predict with certainty what influence, if any, the environment furnished by the medium of Sauton, as modified by Sasano and Medlar, may have on the virulence of B.C.G. It is of importance that the exact status of this question be established by further work.

Dreyer and Vollum,[15] by subculturing two different strains of B.C.G. in large volumes of a simple veal broth-peptone medium, observed definite evidence of pathogenicity. One of the strains had previously been maintained on egg media for several generations and was considered completely avirulent for laboratory animals. Virulence of surprising severity for both rabbits and guinea pigs was observed when subcultures were grown in the depths of the bouillon mentioned. The resultant infection was progressive and was reinoculable. The second strain of B.C.G. investigated had been grown on potato medium prior to coming into the possession of Dreyer and Vollum. Of 56 guinea pigs inoculated with deep bouillon subcultures of the strain, definite progressive tuberculous lesions developed in 5. Although the degree of virulence in the second strain was much inferior to that observed in the first, it nevertheless exhibited a decided increase in pathogenicity after propagation in the depths of the bouillon mixture. Dreyer and Vollum were inclined to believe that the difference in virulence of the two strains was due to factors inherent to the respective microörganisms.

In January, 1930, a strain of B.C.G. (bovine origin) was obtained from Calmette. The organism was growing on glycerine-broth-

potato medium and was continued on a similar medium, prepared in my laboratory, until October, 1930. Subcultures were made every four weeks. Tests for pathogenicity were made by injecting 4 guinea pigs with a suspension prepared from the first subculture made from the original culture obtained from France. At autopsy none of the guinea pigs revealed lesions of a tuberculous nature.

After continuing the culture for nine months on glycerinated broth-potato medium, an experiment was planned to determine what effects a glycerinated egg medium might have on the virulence of the organism.

Methods

Culture Medium: The medium used was a modified formula of that described by Miraglia and consisted of a mixture of egg yolks and 6 per cent glycerinated water.* Transfers were made about the twenty-fifth of each month and the tubes were incubated at 37° C until a luxurious growth appeared. This usually required about two weeks. After a satisfactory growth was obtained the cultures were placed in the refrigerator where they remained until suspensions were prepared for inoculation of animals.

Bacterial Growth: Throughout the period necessary to attain fifteen generations of the organism on the glycerine-egg yolk medium there was no appreciable change in the gross physical character of the culture. It consisted of numerous dry, discrete, grayish white colonies which eventually assumed a slight pinkish tinge. As growth continued those colonies in close proximity became somewhat confluent due to the uneven piling up of the bacterial masses. A diffuse, smooth or spreading type of growth was not observed.

Bacterial Suspensions: Portions of the growth were placed in a

* This medium is prepared as follows: (1) Seven medium-sized, strictly fresh eggs are washed in water and immersed 10 minutes in 80 per cent alcohol. (2) A portion of the shell at one end of the respective eggs is broken away carefully and with sterile, sharp pointed scissors the membranous sac is punctured; after discarding the egg-white, the yolk is discharged into a sterile mixing bowl. (3) To the egg yolks 100 cc. of a 6 per cent glycerine solution prepared as follows is added: glycerine 24 cc., distilled water 500 cc. sterilized in the autoclave for 15 minutes at 15 pounds pressure; the solution is autoclaved in 100 cc. portions and stored for future use. (4) The egg yolks and the glycerine solution are thoroughly mixed with a sterile egg beater and tubed in sterile apparatus. Precautions should be taken to minimize possible contamination. Sterilization is done in the Arnold sterilizer or the inspissator; the first day at 75° C until solidified, then at 85° C for 1 hour, and the second, third, and fourth days at 75° C. Before using, the medium should be incubated for 2 days at 37° C.

sterile mortar and mixed with a few drops of sterile physiological sodium chloride solution. By the use of a pestle a suspension was obtained which was purposely made rather dense. Using additional amounts of physiological sodium chloride solution, a suspension was secured comparable in density to Tube 10 of the McFarland nephelometer. This was permitted to remain for a few hours in the refrigerator to enable the larger clumps to settle to the bottom. The suspension was then ready for use.

Animal Inoculations: With the exception of the first subculture on the glycerine-egg medium, 4 guinea pigs were inoculated from the suspensions prepared from each of the respective subcultures. From the first glycerine-egg subculture 2 guinea pigs were injected, 1 subcutaneously and 1 intraperitoneally. The animals were obtained from our own breeding pens and were usually young adults. Each lot of 4 animals was injected as follows: 2 intracerebrally, each animal receiving 0.4 cc. of the bacterial suspension; 1 subcutaneously, and 1 intraperitoneally, each receiving 1 cc. of the bacterial suspension. The intracerebral injections were made according to a method described previously.[16] After inoculation the guinea pigs were placed two in a cage and housed in a building apart from the structure in which animals used for experimental tuberculosis are kept. They were cared for by an attendant whose duties precluded the possibility of an inadvertent exposure to the germ of tuberculosis, and were fed the regular food provided for the other guinea pigs maintained at the institution.

The inoculated animals were observed daily and dead animals were usually examined either immediately or within a few hours after death. The inoculated animals were permitted to live for a period of from six months to one year after injection unless they died. At the time of autopsy, portions of the principal organs were placed in 10 per cent neutral formalin solution regardless of whether or not there was gross evidence of disease. Sections were prepared from the respective tissues and appropriately stained to reveal the presence of acid-fast bacteria. Attempts were also made to culture acid-fast bacteria that might have been present. Ordinarily only one tissue from each animal was used for cultural purposes. The cultural procedure followed was to treat portions of the emulsified tissue with 5 per cent oxalic acid as recommended by Corper and Uyei [17] and seed the material on glycerinated egg yolk media.

Tuberculin Tests: In a moderate number of instances guinea pigs were injected intradermally with 0.01 cc. of mammalian tuberculin prepared by diluting Koch's old tuberculin with equal parts of physiological sodium chloride solution. The tuberculin tests were usually administered thirty to sixty days subsequent to the inoculations with suspension of B.C.G.

Results

An analysis of data pertaining to the respective guinea pigs in this experiment disclosed that up to March 31, 1932, 43 of the animals died, 11 were killed and 4 are still living (Table I). Among the

Table I

Summary of Results Following the Inoculation of Guinea Pigs with B.C.G.

Intracerebral inoculation	28
Intracerebral inoculation in which tuberculous lesions were found in the brain or the spleen	9
Subcutaneous inoculation	15
Subcutaneous inoculation in which tuberculous lesions developed	1
Intraperitoneal inoculation	15
Intraperitoneal inoculation in which tuberculous lesions developed	1
Total number of animals in which tuberculous lesions were observed (approximately 19 per cent)	11
Animals dying spontaneously	43
Animals killed	11
Animals still living	4
Total	58

animals that died, death occurred subsequent to inoculation as follows: 8 guinea pigs one to thirty days; 11 guinea pigs thirty-one to sixty days; 7 guinea pigs sixty to ninety days; 11 guinea pigs ninety to 180 days, and 6 guinea pigs 181 days to one year. The manner of injection did not seem to have significant bearing on the time elapsing before death.

Although gross lesions of a tuberculous character were not discernible at autopsy, significant lesions were found by subsequent microscopic study in 11 of the animals (Table II); 9 of these 11 had been inoculated intracerebrally, 1 had been inoculated subcutaneously and 1 intraperitoneally. In six instances in which the bacteria had been introduced into the brain, multiple and well defined tuber-

culous lesions were present in the spleen and in the brain.* That a tuberculous infection primary in the substance of the cerebrum may subsequently cause lesions in the spleen was conclusively demon-

TABLE II

Summary of Cases in which Tuberculous Lesions Developed Following Inoculations with B.C.G. All Animals Died Spontaneously

Animal No.	Inoculation	Generation of subculture on glycerine-egg medium	Days before death	Distribution of lesions	Results of culture
11	Intracerebral	4th	14	Brain, liver and spleen	Brain positive
12	Intracerebral	4th	14	Brain and spleen	Brain positive
18	Intraperitoneal	5th	44	Spleen	Spleen positive
23	Intracerebral	7th	14	Brain	Brain positive
24	Intracerebral	7th	31	Brain and spleen	Brain positive
28	Intracerebral	8th	26	Brain, spleen and liver	Brain and spleen positive
51	Intracerebral	14th	38	Brain and spleen	Brain positive
52	Intracerebral	14th	32	Spleen [1]	Brain positive
55	Intracerebral	15th	52	Brain	Brain positive
56	Intracerebral	15th	74	Brain and spleen	Brain positive
57	Subcutaneous	15th	34	Spleen	Spleen positive

[1] Histological sections were prepared from only half of the brain. The other half yielded a culture of acid-fast bacilli.

strated by previous work.[16] Therefore, the observation in this study of tuberculous lesions in the spleen as a consequence of a primary infection of the brain was not considered unusual.

A summary of the autopsy data, pertaining to the 54 guinea pigs that died or were killed subsequent to inoculation with the suspen-

*No lesion was considered tuberculous unless acid-fast bacilli could be demonstrated in suitably stained histological sections, or acid-fast bacilli obtained in cultures prepared from tissue containing the lesions.

sion of B.C.G., shows that 30 of the animals were without demonstrable lesions. The major lesions noted in the remaining animals were: pneumonia, 6 animals; enteritis, 2 animals; purulent adenitis, non-tuberculous focal splenitis, hydrocephalus, peritonitis, hydrothorax, each 1 animal; and tuberculous lesions, 11 animals.

Tuberculin Tests: The attempts to demonstrate an allergic state by the intradermal injection of mammalian tuberculin were without significant results. In only a few instances were suspicious or slight positive reactions observed. Occasionally a small area of marked induration occurred, but in no instance was a typically positive reaction obtained.

Pathological Histology

The lesions of the brains of the animals that died within two weeks were usually confined to the sulci, although in a few instances there was a limited involvement of the pia mater (Fig. 1). Brains of animals in which the infection had existed for a longer period revealed lesions that extended rather diffusely into the depths of the cerebral tissue (Fig. 2). The lesions, which were composed largely of monocytic cells, had a perivascular inception and were not circumscribed or encapsulated. Infrequently lesions were observed in which caseation necrosis was beginning. Giant cells of the Langhan's type were seldom seen, and the relatively short duration of the disease apparently seemed to have precluded the deposition of mineral salts in the necrotic areas. Acid-fast bacilli were numerous in practically all of the lesions of the brain. The monocytic cells for the most part retained their immature characteristics and in only one instance did the cells show a tendency to assume an epithelioid appearance. This was observed in the brain of Guinea pig 55, which died fifty-two days after inoculation.

The splenic lesions were multiple. They began in the splenic corpuscles and many extended peripherally to the outer confines of these structures, replacing the lymphoid elements as a consequence. The splenic pulp was seldom violated (Fig. 3). Encapsulation was not apparent and only a minimal amount of necrosis had occurred. The cellular elements constituting the respective lesions were mainly monocytic, but unlike those observed in the brain there was a marked tendency for many of the cells to assume an epithelioid appearance (Fig. 4). Giant cells of the Langhan's type were too infre-

quent to be significant. Many acid-fast bacilli were demonstrable among the cells of the splenic lesions, although for the most part the organisms were not so numerous as in the cerebral tissues.

The lesions of both the brain and the spleen were indistinguishable from those of comparable duration in these organs following the experimental introduction of virulent mammalian strains of *Mycobacterium tuberculosis*. The histopathology was typically that of an early progressive tuberculous process with little if any evidence of a significant inhibitory mechanism.

Reinoculation of Infective Material

Several attempts were made to transmit the disease to other animals by emulsions of tissue of animals that had died, or by pure cultures of acid-fast bacilli obtained from the organs of animals possessing lesions of a significant character. Generous amounts of the inoculum containing enormous numbers of acid-fast bacilli were used for inoculation and in most instances rabbits, in addition to guinea pigs, were inoculated. The guinea pigs were inoculated either subcutaneously or intraperitoneally, and the rabbits intravenously. Although a few of the animals died within the first sixty days after inoculation the greater number survived to be killed at the expiration of six to eight months.* At the time of writing, none of these attempts to induce lesions of tuberculosis by injecting into animals viable acid-fast organisms obtained from lesions of guinea pigs previously inoculated with B.C.G. has been successful.

Comment

The data obtained from these experiments do not afford evidence that the particular B.C.G. strain used has experienced a progressive increase in pathogenicity sufficient to account for the tuberculous lesions that were found. The bacteria that incited lesions were obtained from glycerine-egg media, Subcultures Nos. 4, 5, 7, 8, 14 and 15; yet the animals inoculated with Subcultures Nos. 1, 2, 3, 6, 9, 10, 11, 12 and 13 were without demonstrable lesions of tuberculosis. It is worthy of note that not all of the animals that were inoculated manifested tuberculous lesions even though one or two may have.

* Several animals are still under observation.

One might expect the intracerebral route of inoculation to be a more vulnerable portal of entry than the intraperitoneal or subcutaneous route; therefore it seems most unusual that lesions should develop in one animal of a group after subcutaneous or intraperitoneal injection, and that lesions had not developed in one or both of the animals that received portions of the same suspension intracerebrally when examined at autopsy months later. This happened in the case of the guinea pig given injections of the suspension prepared from the fifth subculture of the organism grown on the glycerine-egg medium. Animal No. 18, injected intraperitoneally February 12, 1931, died forty-four days later with definite tuberculous lesions in the spleen from which a culture of acid-fast bacilli was obtained, yet in none of the other 3 animals in this group were lesions established that could be demonstrated either grossly or microscopically. One of the animals given intracerebral injections was living almost a year later and was finally killed for autopsy.

Individual susceptibility, rather than a general increase in the virulence of the organism, would seem the most logical explanation for the occurrence of the tuberculous changes observed in this study. The general physical condition of the respective animals in which lesions were found was not below that which obtained for cage mates that were devoid of lesions. The resistant state of the animals did not appear to be influenced by, or to be dependent on, factors pertaining to food, care or environment. In fact, the animals in which lesions occurred were generally in good, if not excellent, physical condition at the time of death, as judged by the plumpness of musculature. The reason why lesions developed in some animals and not in others cannot be explained satisfactorily.

When one considers the length of time Calmette and his collaborators cultured B.C.G. on the glycerinated bile-potato medium before the organism attained its present state of reduced virulence, it is not surprising that marked reversion did not occur after subculturing for fifteen generations on the glycerine-egg medium. It would seem logical to assume, however, that since a diminution of virulence occurred by prolonged cultivation on the virulence-reducing glycerine-ox bile-potato medium, an extended tenure on a favorable medium might bring about restoration of the initial virulent state. This hypothesis justifies a continuation of this work.

Failure to establish a progressive tuberculous infection by the re-

inoculation of animals with infective material obtained from guinea pigs with definite tuberculous lesions provides an illustration which invalidates the conclusion that the virulence of the particular strain of B.C.G. used in this study has materially increased as a consequence of a prolonged residence on the glycerine-egg medium. Until it is possible consistently to promote tuberculous lesions by the reinoculation of such material, the pathogenic propensities of the organism remain problematic. On the other hand, to assert that the bacteria are entirely innocuous would hardly be acceptable on the basis of unquestionable evidence of pathogenicity in at least 11 of the guinea pigs. It is true that rather large doses of the bacterial suspensions were injected into the respective animals, but the subsequent development of tuberculous lesions in approximately 19 per cent of the guinea pigs inoculated is hardly a negligible circumstance. It seems pertinent to note also that inability to infect additional animals by the reinoculation of material from these lesions did not appreciably mitigate the severity of a disease that was characterized by the formation of definite tuberculous lesions and the eventual death of the respective animals. Whether or not it is possible to induce progressive tuberculosis that is reinoculable, the fact remains that the particular strain of B.C.G. studied, when introduced into guinea pigs in large amounts, not infrequently incited a tissue reaction of a tuberculous character that apparently resulted in death. In other words, simply because the disease was not transmissible by reinoculation did not make the lesions any less real in the respective animals in which lesions were found.

This study emphasizes the necessity of the examination of properly stained histological sections before a decision is made on the tuberculous or non-tuberculous content of a tissue. Decisions based on the gross appearance of a given tissue are frequently fallacious. In none of the tissues observed in this study could gross evidence of tuberculosis be seen, yet definite tuberculous lesions were eventually found in 11 of the animals. In work of this kind tissues should be preserved from every animal at autopsy in order that significant lesions may not be overlooked.

Summary and Conclusions

Using a strain of B.C.G. obtained from Calmette of the Pasteur Institute, a deliberate attempt was made to increase its pathogenicity by subculturing the organism on a glycerinated egg medium. Transfers were made every thirty days. From each succeeding subculture 4 guinea pigs were given injections — 2 intracerebrally, 1 subcutaneously, and 1 intraperitoneally. The report deals with data obtained after the organism had been subcultured on glycerinated egg medium for fifteen generations.

Of a total of 58 guinea pigs inoculated, lesions histologically indistinguishable from those of genuine tuberculosis occurred in the tissues of 11, and cultures of acid-fast bacilli were obtained from each. Although the majority of the lesions occurred in animals that had been given intracerebral injections, 1 animal that was given an intraperitoneal injection and another given a subcutaneous injection died with lesions of a tuberculous nature. So far, attempts have failed to promote a succession of tuberculous lesions by the reinoculation into guinea pigs of infective material from lesions.

The particular strain of B.C.G. studied is not devoid of pathogenicity for guinea pigs, and the assertion that the organism is innocuous cannot be accepted without reservations.

Subculturing the organism on glycerinated egg medium at monthly intervals for a period of fifteen generations did not markedly enhance its virulence.

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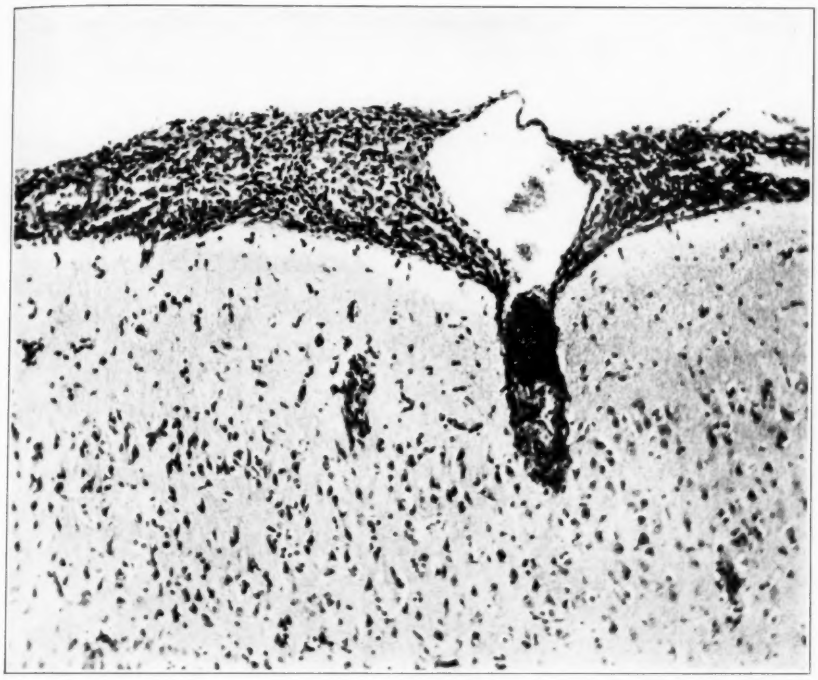
DESCRIPTION OF PLATES

PLATE 119

FIG. 1. Cerebral meningitis of Guinea pig 55. Animal died fifty-two days after intracerebral injection of a suspension of B.C.G. Culture was the fifteenth generation grown on glycerinated egg medium. Lesion is essentially a monocytic reaction. Acid-fast bacilli were present in the lesion. × 130.

FIG. 2. Multiple lesions in the brain of Guinea pig 28. Animal died twenty-six days after intracerebral injection of a suspension of B.C.G. Culture was the eighth generation grown on glycerinated egg medium. Many acid-fast bacteria were present in the cerebral lesions and the spleen was also affected (see Fig. 3). × 140.



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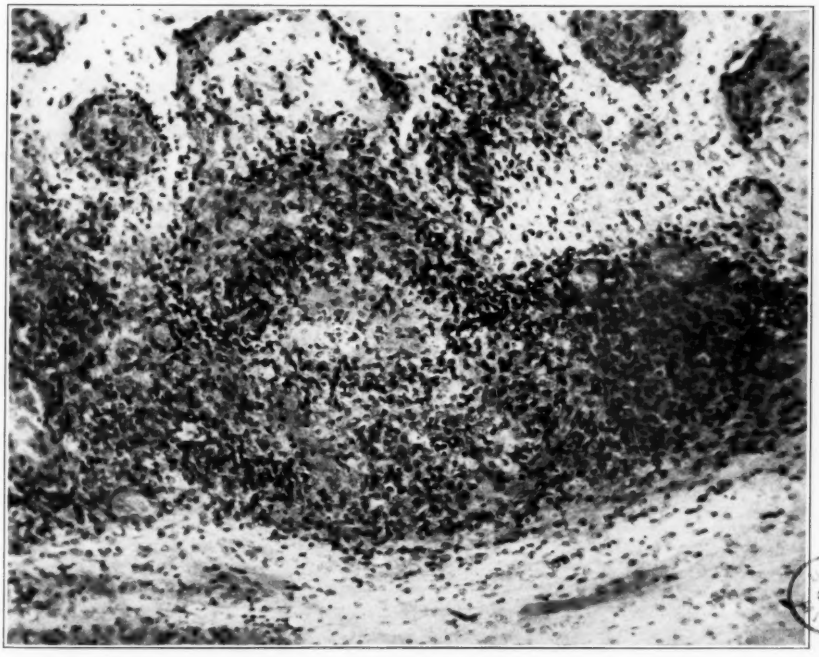

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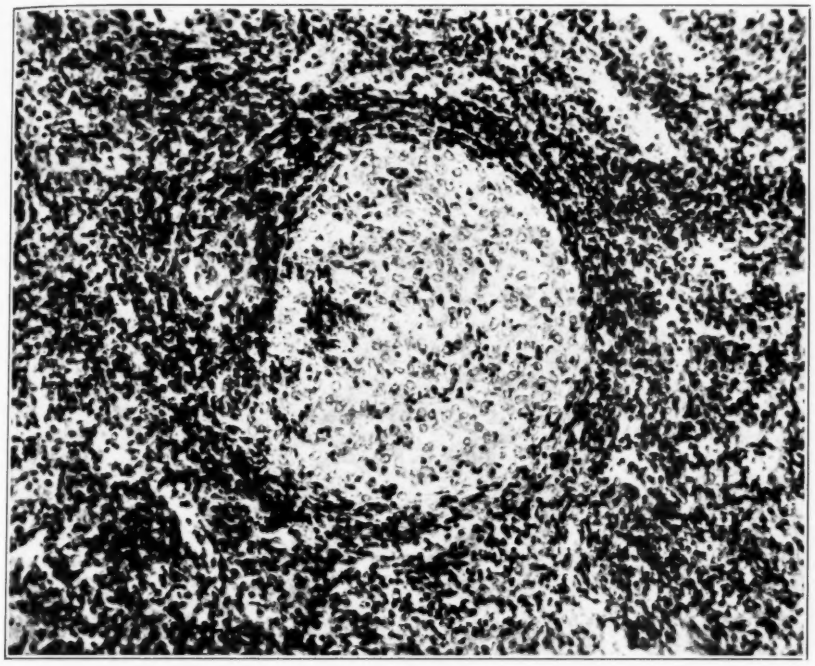
PLATE 120

FIG. 3. Spleen of Guinea pig 28. The splenic lesions were multiple and were composed largely of epithelioid cells. A culture of acid-fast bacilli was obtained from an emulsion prepared from a portion of this spleen. × 170.

FIG. 4. Focal lesion consisting largely of epithelioid cells occupying a splenic nodule (Guinea pig 18). Animal died forty-four days after intraperitoneal injection of B.C.G. The culture was the fifth generation on glycerinated egg medium. Cultures of acid-fast bacilli were obtained from an emulsion prepared from a portion of this spleen. × 120.



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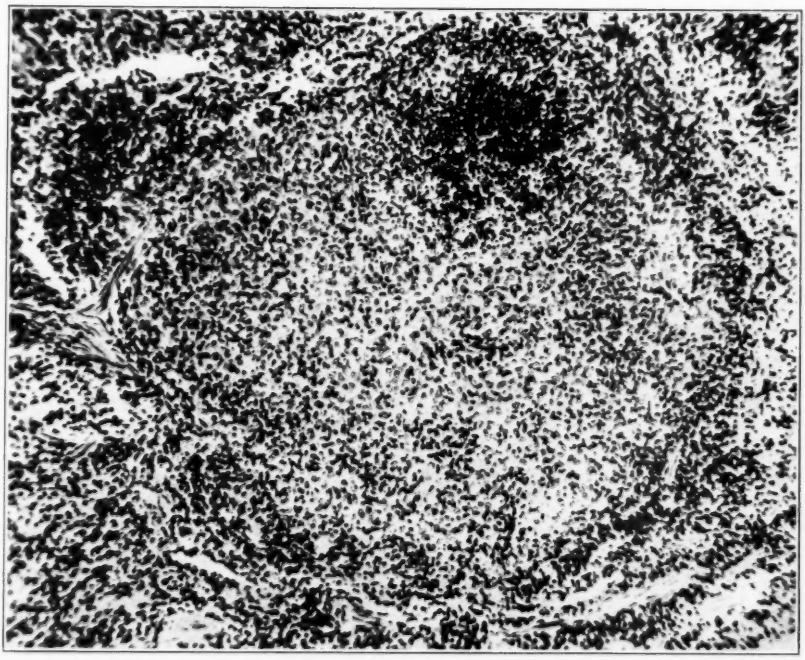

4

TWO SIMPLE METHODS FOR THE SILVER IMPREGNATION OF NERVE FIBERS IN PARAFFIN SECTIONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM *

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Most pathologists will agree that the methods hitherto devised for the silver impregnation of nerve fibers have been of such a nature as to inspire a certain degree of hesitancy, not to say awe, in the average laboratory worker. Up to the present time it appears that there is but one method available for the silver impregnation of nerves in paraffin sections, that of Rogers,[1] which is of recent date; otherwise one has been thrown back on block or frozen section impregnations which require a certain amount of skill and practice for their execution, and have the tendency to be somewhat capricious and uneven and, hence, to intimidate the beginner in silver technique.

In this paper two methods are described that afford the opportunity for impregnating nerve fibers and fibrils in the brain, cord and peripheral nerve trunks under ordinary circumstances of paraffin sectioning; one of them is a modification of the Ramon y Cajal block impregnation and the other is a practically unmodified Rogers technique. Rogers designed this for the demonstration of non-medullated nerve terminals, especially motor endings. All that has been done here is to apply his procedure to the central nervous system and to try a few experiments with fixatives that he had not mentioned.

THE SILVER NITRATE METHOD

Fixation: After experimenting with about eight different types of fixative, it has been found that the best results are obtainable with a Carnoy's solution that contains no mercury and is made up of absolute alcohol 6 parts, chloroform 3 parts and glacial acetic acid 1 part. The chloroform and alcohol may be kept mixed and the acid added in the proper proportion when it is desired to fix tissue, other-

* Received for publication April 11, 1932.

wise the mixture becomes rapidly converted into ethyl acetate if the acid be added immediately and the fluid kept in stock. This fixative dissolves out the lipids from the nervous tissue and leaves the nerve fibers slightly shrunken but of uniform caliber and with an advantageous index of refraction, so that they stand out clearly. The tissue is fixed for 24 hours, transferred to absolute alcohol for an hour or so and then run through chloroform and chloroform-paraffin into paraffin.

Pretreatment: The sections are deparaffinized with xylol and absolute alcohol in the usual way and they are then left for 1 to 12 hours in a mixture of pyridin 2 parts to glycerol 1 part. They are then washed in 95 per cent alcohol, followed by distilled water, to remove most of the pyridin. A trace of this does not appear to make any difference in the ultimate result.

Impregnation: The slides are then immersed in 10 per cent aqueous silver nitrate and the staining box set on the warm-plate of an incubator run at 37°C for 12 hours or so, the box being covered to prevent evaporation. They are then washed in 2 changes of distilled water. The impregnating fluid may be used repeatedly and kept in the incubator.

Development: The sections are developed for 20 minutes in a 5 per cent neutral formalin solution containing 0.5 per cent pyrogallol, in which they turn yellowish brown. They are then washed at the tap. The developer is prepared fresh for each box of slides.

Toning: Two toning solutions are available: for general purposes a 1:500 aqueous gold chloride is used; added nuclear precision with less intense glial impregnation is obtained if 2 per cent glacial acetic acid be added to this. Five minutes' toning is sufficient to replace silver with gold. The solution keeps for some time and may be used until its effects are visibly weakening.

Intensifying: This important step, devised by Laidlaw,[2] consists in further reducing the gold by means of immersion for 5 minutes in oxalic acid; in this case it has been found best to add formalin, so that a 1 per cent neutral formalin solution contains 2 per cent oxalic acid. The sections, which have become blackish, grayish or brownish in the toning bath, turn a pleasant violet in this intensifier. They are then well washed at the tap, to prevent precipitating out sulphur from the hypo bath that follows. The intensifying bath may be used repeatedly over long periods of time.

Fixing and Mounting: The sections are fixed for 5 minutes in a 5 per cent aqueous solution of sodium thiosulphate, washed at the tap, dehydrated in ascending percentages of alcohol, run through xylol and mounted in Canada balsam.

The Rogers Technique

Fixation: Neutral formalin has been found to be the best, which is a great advantage to the routine pathologist; the fixative employed in the preceding method may also be used. Rogers recommends dehydrating the tissue, after washing, in ascending percentages of alcohol to which 1 to 2 per cent of strong ammonia has been added, then placing it in pure absolute alcohol for a few hours and embedding as usual. While this gives excellent results in mixed tissues, it is found to cause some swelling and distortion in brain sections and it is not necessary in any event for the demonstration of nerve fibers unless their end-plates are to be brought out. The modified Carnoy fixative gives good results where coarser, medullated fibers are to be shown; neutral formalin being better for finer, non-medullated fibers.

Pretreatment: After deparaffinizing, the sections are placed in 95 per cent alcohol with 2 per cent strong ammonia for 12 hours or longer. This step is very important as it alkalinizes the sections, which are then rinsed briefly in 80 per cent alcohol and transferred directly to body-warm 40 per cent aqueous silver nitrate for 20 minutes in the incubator. This fluid may be used repeatedly over long periods if it is kept covered to prevent evaporation and contamination.

Combined Impregnation and Development: The sections are next briefly rinsed in distilled water and flooded with 20 per cent neutral formalin for 5 minutes, coming from this to 5 per cent neutral formalin. The staining box is then set on a table, another one is filled with *fresh* 20 per cent neutral formalin and placed nearby and a third is inverted and set between the two. The sections are removed one by one from the weak formalin, blotted briefly on filter paper to remove excess formalin and laid on the inverted box, where they are flooded with a few drops of diammoniacal silver from a dropping-bottle. This solution is prepared by adding strong ammonia drop by drop to 20 cc. of 20 per cent silver nitrate solution until the resulting precipitate is just dissolved. Then an excess of 10 drops of ammonia

is added and the water-clear mixture diluted with 20 cc. of distilled water and poured into a 50 cc. dropping-bottle where it may be kept until it is used up.

Three slides fill the space on the inverted staining box and after each has been flooded in turn with 4 to 5 drops of the silver solution, the first one is blotted and placed directly in the 20 per cent formalin, while another section is removed from the weak formalin, blotted, flooded with diammoniacal silver and left in the place of the first until the turn comes round. The second section is then removed, blotted, flooded and set in the strong formalin with the first and so on until all the sections have been treated in rotation and are in the developer where they turn rusty orange, and where they should remain another 5 minutes to ensure complete development. It is found that the time required to flood, blot and transfer three sections in this manner is just sufficient for proper impregnation — about 1 minute in practice.

Subsequent Treatment: Toning is accomplished by 15 minutes' treatment in a 1:300 gold chloride bath, acidified with 2 per cent glacial acetic acid. Intensification, fixing, dehydration and mounting are precisely similar to that which has been described in the case of the preceding method.

Precautions: The reader is cautioned that the diammoniacal silver solution is made up *without the addition of sodium hydroxid or sodium carbonate* usually practised in Bielschowsky or other methods. In carrying out the Rogers method one is dealing with very concentrated silver solutions and should, therefore, protect the hands with rubber gloves and the clothing and shoes as well from the action of these. Rubber gloves, old shoes and a capacious laboratory apron or coat can not be too highly recommended.

Brief Resumé of Steps

Silver Nitrate Method

1. Pretreatment with pyridin-glycerol for 1 to 12 hours.
2. Wash in 95 per cent alcohol followed by distilled water.
3. Impregnate 12 hours or longer in 10 per cent silver nitrate in incubator.
4. Wash in 2 changes of distilled water.
5. Develop in pyrogallol-formol 20 minutes.

6. Wash at tap and tone 5 minutes in gold bath.

7. Intensify in oxalic acid-formalin 5 minutes after washing at tap.

8. Wash well at tap and fix in hypo for 5 minutes.

9. Wash, dehydrate, clear and mount in Canada balsam.

Rogers' Method

1. Pretreatment with ammoniated alcohol 12 hours or more.

2. Rinse in 80 per cent alcohol and impregnate in 40 per cent silver nitrate in the incubator for 20 minutes. Rinse in distilled water.

3. Treat with 20 per cent formalin 5 minutes, transfer to 5 per cent formalin.

4. Reimpregnate sections one by one, after blotting off formalin, with diammoniacal silver; blot and develop. Do not wash!

5. Develop in 20 per cent formalin 5 minutes, or until all sections are a rusty orange.

6. Wash in distilled water and tone in gold bath for 15 minutes.

7. Wash at tap and intensify, fix and mount as in preceding schedule.

Remarks

With these two methods at one's disposal a great deal can be done in connection with the study of nerve fibers of different types. For the brain and cord (Fig. 1), as well as for large peripheral nerve trunks like the sciatic or femoral (Fig. 2), the silver nitrate procedure is best adapted; for the demonstration of the finest neurofibrillae, such as the non-medullated fibers in the nuclei of the medulla or those in the superficial cortex (Figs. 3 and 4) the Rogers method is the better.

The silver nitrate technique is about as simple as a silver impregnation could be; it gives a clear-cut impregnation of the nerve fibers which take on a light red or fox-red color and stand out from the very fine, violaceous glia fibrils. The nerve cells, ganglion cells and the like, tend to take on the same reddish tone as do the neurones; the neuroglia cells, particularly the oligodendrogliocytes, impregnate grayish to blackish. This fact may conceivably be of use in certain investigations. The method is not suitable, nor is it intended for the demonstration of neuroglia cells and their processes.

On the other hand, it impregnates nerve fibers very successfully and few of them elude it; the basket fibers around Purkinje cells and the fibers in the granular zone of the cerebellum are best brought out in sections toned with acid gold. The nuclear detail in the cells is very good; if they tend to overimpregnate, the acid gold bath will correct this at the cost of slight loss of general intensity of the fiber impregnation. Although this method impregnates the finest fibrils in sections of nervous tissue, they are apt to be obscured somewhat by the presence of neuroglia fibers. In this case, one resorts to Rogers' method which is more selective for the finer elements.

In that method these are brought out black on a less densely impregnated background, the details of the cellular elements and of their nuclei may not be as sharply picked out as in the preceding method, but they may be very adequately shown. Following fixation in the modified Carnoy solution Rogers' method gives good impregnation of the coarser fibers, the color scheme tending to be more on the red, less on the black side. The reader may experiment with the two methods and ascertain which of them suits his particular needs.

It will be noted that, after repeatedly stressing the importance of using equimolar solutions I have not employed them here; the reason is quite simple — they do not work as well as do the concentrations herein noted, which are the result of considerable experimentation. Naturally, this does not apply to the Rogers method which has been taken over practically unchanged.

Summary

Two methods are described for impregnating nerve fibers in paraffin sections; one is a modification of the Ramon y Cajal silver nitrate block impregnation, while the other is a practically unmodified Rogers technique. The former is best suited to the demonstration of fibers in the central nervous system and in large peripheral nerve trunks in their relation to supporting structures: the latter is an application of Rogers' method for demonstrating terminal nerve endings for the more general purpose of bringing out non-medullated fibrils in the central nervous system. Both methods are designed for general use in the histological laboratory on tissue that has been fixed not over five hours postmortem. The silver

nitrate method is well standardized and should produce reasonably uniform results; the Rogers method may entail a modicum of practice in the step whereby the sections are reimpregnated one by one in the diammoniacal silver, otherwise it presents no pitfalls for the unwary.

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DESCRIPTION OF PLATE

PLATE 121

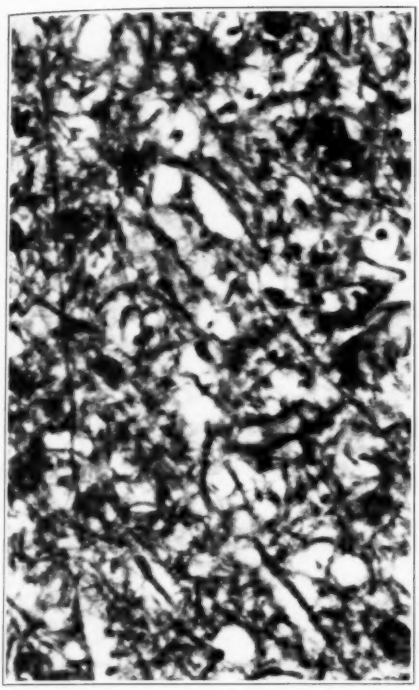
All photomicrographs were taken at 800 diameters by Prof. J. B. Homan of our Department of Medical Art, with the assistance of the author.

FIG. 1. Silver nitrate method, fibers in cortical white matter.

FIG. 2. Silver nitrate method, fibers in the femoral nerve. Most of the coagulation phenomena seen in sections fixed by other methods that do not extract the myelin are done away with here. The "funnels" remain.

FIG. 3. Rogers' method. Fine fibrils in gray matter of cortex.

FIG. 4. Ganglion cells and fine fibrils from the region of the floor of the fourth ventricle. Rogers' technique.



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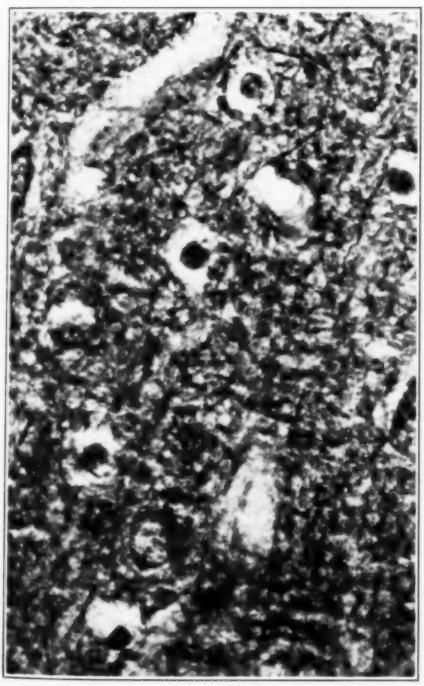

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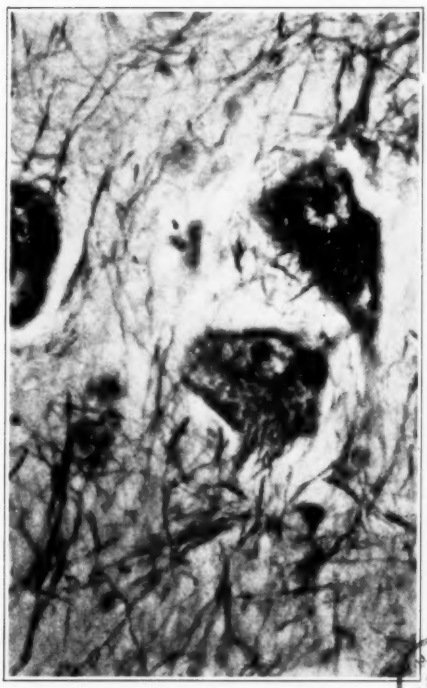

4

Silver Impregnation of Nerve Fibers

THE EFFECT OF DIFFERENT TYPES OF FIXATION ON THE SILVER IMPREGNATION OF PARAFFIN SECTIONS OF PERIPHERAL NERVE *

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While experimenting with a method for impregnating paraffin sections of nervous tissue with silver, as already reported elsewhere (Foot [1]), it was found that the results obtained in the case of peripheral nerve trunks varied widely with the fixative used. So wide was this variation and so striking, that it is thought to be of sufficient interest to be reported. Some work has been done along this line by Davenport,[2] who experimented upon blocks of cat's spinal cord, using a variety of fixatives and a standard silver nitrate impregnation (Ramon y Cajal), and bending his efforts toward determining the most favorable ammonia concentration in the alcoholic fixing fluid, together with the effect of extraction of lipins from the tissue by means of adjuvants to the alcohol, such as chloroform and pyridin. He found that the optimum impregnation was obtained after the use of alkaline fixatives that, at the same time, extracted the lipins and thus permitted complete penetration of the silver salt. Furthermore, he investigated the effect of varying the proportion of pyrogallol and formalin in the developer and found the former to be the essential ingredient, as formalin did not reduce silver nitrate although it does reduce the double ammonia salts of silver. His second paper [3] dealt with acid fixatives, such as sulphosalicylic acid, trichloracetic acid, Hofker's fluid and Carnoy's fixative. There was less shrinkage of the tissue than was the case when ammoniated alcohol had been used, but during the necessary alkalinization, washing and silvering, the acid-fixed tissue ultimately shrank more than the controls, Carnoy's solution bringing about the maximum shrinkage noted. This he determined by measuring the blocks before and after fixing and impregnating. Successful silver impregnation was obtained after the use of all the fixatives with which he experi-

* Received for publication May 26, 1932.

mented, which is of interest because it is not generally known that silver impregnation will succeed after fixation in other than one or two of the commoner formalin or bichromate methods. It was to determine the possible range of fixatives at one's disposal that the work reported in this paper was undertaken prior to reading Davenport's articles.

Technique

The essential for this experiment was to have a standard material and a standard impregnation that would be as simple and constant as possible. Accordingly, a few millimeters of femoral nerve was removed not later than two hours postmortem from human subjects, while they were still warm. As the impregnation, an adaptation of the familiar Ramon y Cajal block impregnation was used, in which the tissue was first sectioned in paraffin, mounted on slides and deparaffinized in the usual manner, after which the sections were treated with pyridin 2 parts, and glycerol 1 part, for 1 to 12 hours. They were then washed in 95 per cent alcohol, followed by tap water and 2 changes of distilled water, and impregnated for 12 to 24 hours in a 10 per cent aqueous silver nitrate solution in the incubator at 37° C.

After a wash in distilled water they were developed for 20 minutes in a mixture of pyrogallol and formalin, 5 parts each to 100 parts distilled water. It was later found that the pyrogallol could be reduced to one-tenth this concentration without materially changing the picture. After developing, the sections were washed at the tap and toned for 5 minutes in a 1: 500 aqueous solution of Merck's "acid red" gold chlorid. They were again washed at the tap and intensified for 5 minutes in a mixture containing oxalic acid 2 per cent and formalin 1 per cent in distilled water, after which they were well washed at the tap and fixed in 5 per cent sodium thiosulphate for 5 minutes, washed and then dehydrated, cleared and mounted in Canada balsam in the usual way.

Fixatives and Results

The fixatives used may be grouped into four general classes:

(1) *Alcoholic Fixatives:* (*a*) 95 per cent alcohol; (*b*) absolute alcohol; (*c*) ammoniated absolute alcohol, containing 2 per cent

strong ammonium hydroxid; (*d*) alcohol 6 parts, chloroform 3 parts and glacial acetic acid 1 part (omitting the mercury bichloride that is usually an ingredient of Carnoy's solution).

(2) *Formalin Fixatives:* (*a*) Neutral 10 per cent formalin, and (*b*) Ramon y Cajal's "bromformol" with 15 per cent formalin and 3.5 per cent ammonium bromid.

(3) *Chromic Fixatives:* (*a*) Zenker's fluid, with 5 per cent mercuric chlorid, 2.5 per cent potassium bichromate and no sodium sulphate, adding 5 cc. of glacial acetic acid to every 95 cc. of the mixture at the time the blocks are fixed; (*b*) Helley's fluid, which is identical with Zenker's except for the substitution of 10 cc. of strong formalin in the place of the 5 cc. of acetic acid and the reduction of the amount of stock solution from 95 cc. to 90 cc.

(4) *Acid Fixatives:* This group was represented by Bouin's solution (water-saturated picric acid 15 parts, strong formalin 5 parts and glacial acetic acid 1 part).

Alcoholic Fixation: Nerve trunk fixed in the alcoholic solutions shows the axones to be somewhat shrunken, but of a nearly uniform caliber. They are reddish to yellowish brown and stand out with remarkable distinctness. The rather complicated pictures to be described later in connection with the myelin sheaths are not noted; there is an outer envelope with a space that contains a few bits of granular, or globular débris and the shrunken funnels of Schmidt, which are usually collapsed about the neurone as dark swellings. In the modified Carnoy's fixative, however, they are more clearly brought out and less shrivelled. The nodes of Ranvier are well demonstrated. There is a marked simplicity to the histological picture, which is to be attributed to the extraction of coagulable or argentaffin lipins from the myelin sheath. One may find areas where extraction has been less complete and where bubble-like outlines and granular masses may be found to be impregnated. In those portions of the section where the extraction has run its course, this layer of the sheath is almost clear and empty, save for the Schwann cells. The connective tissue elements are not particularly well impregnated and tend to be slaty or violaceous in color, which is an advantage in that the neurones stand out in contrast by reason of their brownish tint (Fig. 1).

The leashes of delicate fibrils that parallel the neurones in the nerve trunk and lie between them take on the same brownish hue

as do the neurones, which is also true of the fine fibrils that are embedded in the connective tissue of the epineurium. The nuclei of the tissue are all well shown. There is not much fundamental difference between the fixatives in this group, excepting that the modified Carnoy's solution is the most successful while the 95 per cent alcohol gives poor results. The former, containing acetic acid, tends to offset the shrinkage of the fibers that results in straight alcohol fixation. There is, therefore, a graded scale of excellence between the modified Carnoy's solution at the top and stock, unalkalinized alcohol at the bottom.

Formalin Fixation: Neutral formalin is commonly supposed to be the ideal fixative for silver impregnations, but the results obtained in this experiment with formalin alone, or in combination with ammonium bromid or other ingredients, are not in accord with this conception in so far as peripheral nerves are concerned. One sees at once that the axones show very marked irregularity of caliber and outline (Fig. 2), although they are metachromatically impregnated and stand out prominently by reason of their fox-red color. Here and there one notes fusiform, or beaded swellings of the axones, in which the substance of the fibers is pale; elsewhere the neurones may be markedly contracted and delicate. Thus the fibers show an alternation between what may be considered quasi normal caliber and either fusiform swelling, or marked shrinkage. The perineural fibers are well brought out, as is the connective tissue of the nerve trunk. The nuclei are beautifully impregnated, the funnels stand out well and there is a wealth of detail in the myelin sheath, which stains yellowish to orange. One finds longitudinal fibers and a very intricate, wide-meshed web of tiny fibers that radiate through the sheath and make a very pleasing, though probably deceptive picture. In the case of the formalin-bromid fixative the connective tissue becomes too prominent and darkly impregnated, the neurones are even more irregular in caliber and there is an orange network in the myelin sheath, as in the preceding case. The reticulum of the fibrous sheath and of its vessels is well demonstrated, in sharp contrast to the alcohol-fixed specimens.

Chromic Fixation: In this case one would think that one was dealing with quite another histological structure, so strikingly different is the picture obtained (Fig. 3). The myelin sheath has been impregnated a reddish brown, it shows innumerable radiating, frond-like

fibrils that appear to spring from the pinkish neurone and run out to the sheath of Schwann. The funnels are embedded in this network and are almost indistinguishable, the neurones are of fairly even caliber, pinkish color and very well and evenly brought out, while the connective tissue of the sheaths is violaceous to grayish. In cross-sections of nerve fibers the familiar "Sonnenbilder," or rosettes, are prominent. The picture is very pleasing and appears to be very accurate at first glance, but one may well believe that it is, on the contrary, quite deceptive.

In the chromium-mercury methods one is forced to use iodin and sodium thiosulphate to remove the mercuric chlorid crystals, before proceeding with the impregnation. Experiments with sections fixed in other types of solution failed to produce similar pictures if iodin and hypo were used, so that we may suppose that the effect noted in the myelin sheath is due to the action of chromium or mercury and not to the iodin treatment. Chromicizing formalin- or alcohol-fixed sections for 24 hours in the incubator with a mixture of equal parts of 10 per cent chromic acid and saturated aqueous bichromate of potash fails to produce similar pictures, however, so that these salts must, apparently, exert their effect only on fresh, unfixed tissue. It should be noted, however, that formalin fixation produces somewhat similar pictures; it seems as though the coagulative action in this case does not proceed as far, or act so strongly.

Acid Fixation: Material fixed in Bouin's solution gives results that accentuate connective tissue elements at the expense of the nervous. The neurones are irregularly, often very lightly impregnated (Fig. 4) and there is more swelling and distortion of these than was the case after formalin fixation, long stretches of nerve fiber being swollen to the capacity of the sheath and of greater diameter than in any other case. The illustration shows this only in part. They are often so faintly impregnated that they are difficult of identification. The nuclear and connective tissue detail is very good.

Review and Discussion of the Findings

Reviewing these results we find that the most reliable fixation for the purpose of silver nitrate impregnation corresponds with Davenport's experience — it should be one that extracts the lipins and permits the penetration of the silver solution. The disadvantage of

alcohol is its tendency to shrink the axones, but this may be offset by the addition of acetic acid. The use of such a fixative demonstrates the defects of fixatives that may combine with, or coagulate the lipins — for in such a case histological details are brought out that probably do not exist in fresh tissue.

It is surprising to find formalin combinations so disturbing to the neural histology in view of their extensive use in fixing nervous tissue and in connection with silver techniques; it is disappointing to discover how much they distort the actual nerve fibers while they give such excellent results in the case of their adnexae. The more concentrated formalin in Ramon y Cajal's fixative seems to increase this distortion and this is not corrected by adding picric and acetic acid, and decreasing the percentage of formalin, as in Bouin's solution. Further proof of this action on the part of formalin may be obtained through the observation of nerve fibers impregnated by Rogers' method,[4] in which the impregnation is very fine but the outline of the neurones actually scalloped, after fixation in formalin or Bouin's fluid. One immediately wonders how accurate are the pictures produced in non-medullated nerves and neural end-organs under such circumstances.

The optimum fixative, then, would be one which would give: (*a*) as little distortion of the nerve fibers as possible; (*b*) metachromatic results, so that nerves and connective tissue fibers have different colors; (*c*) good nuclear impregnation and (*d*) good impregnation of the connective tissue elements without unduly emphasizing them. Thus far, in connection with this simple silver nitrate impregnation, the alcohol-chloroform-acetic acid mixture seems most nearly to accomplish these ends.

Supplementary Work

As it would be interesting to determine what result, if any, would be obtained through the use of Mallory's preliminary "bleach" of permanganate of potash and oxalic acid, sections fixed in the four types of fixative were subjected to treatment with weak alcoholic iodin, weak sodium thiosulphate to remove this and then treated with 0.25 per cent aqueous potassium permanganate, followed by 5 per cent oxalic acid. They remained in each of these solutions for 3 minutes with washes in water between each step. It was found that the bleach almost invariably tends to loosen the sections from

the slides, unless they have been very carefully mounted and dried. This has been a troublesome feature often noted in reticulum impregnation and it is probable that it may be overcome by the use of Masson's formalin-hardened gelatin method of mounting sections.[5]

The net result after using the bleach is not very striking; the sections show some increase in precision of detail at the expense of depth of impregnation. The Zenker-fixed sections show much more delicate pictures, except that they have become monochrome (brownish) without the desirable metachromasia. The Bouin-fixed sections are even worse than before. It would seem that there is nothing to gain and much to lose through the use of the bleach in this particular connection. It is to be noted that this does not apply to connective tissue impregnations, merely to those of nerve trunks.

Summary

1. Experiments on four representative groups of fixatives, used on standard material (normal human femoral nerve) and followed by a standardized and very simple method of silver impregnation, show that the results differ almost directly as the number of fixatives used. Although the variation within a given group of similar fixatives is slight, those between any two groups are decidedly marked.

2. Alcohol fixation tends to remove lipins from the myelin sheath and thus affords the clearest impregnation, possibly the most veracious. The best alcoholic solution is a combination of alcohol, chloroform and acetic acid.

3. Formalin fixation, even when neutral formalin is used, causes marked distortion of the neurones and brings out a certain amount of what may be considered to be extraneous detail, caused by the coagulation or chemical alteration of the myelin.

4. Chromate fixation, while it affords very precise and clear pictures, demonstrates even more histological detail, which is probably artefact, owing to a similar, but more pronounced action on the lipins.

5. Fixation in such an acid solution as Bouin's fluid causes an accentuation of the connective tissue elements at the expense of the nervous and gives to the neurones an unduly swollen and transparent appearance without effecting much metachromatic contrast.

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DESCRIPTION OF PLATE

PLATE 122

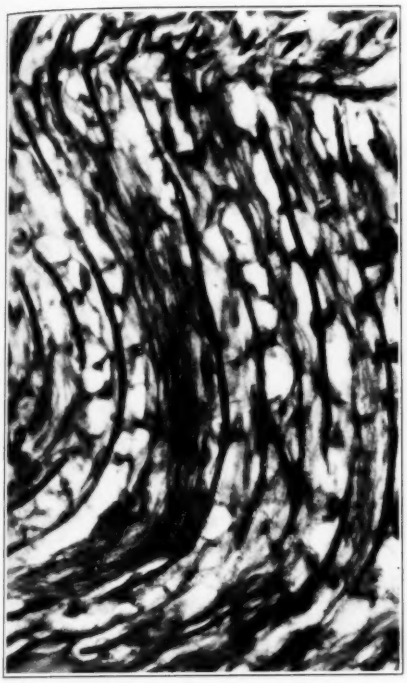
All four photomicrographs were made from sections of human femoral nerve, removed while the body was still warm. The impregnation is identical in each case, the only difference being in the fixative employed. The magnification represents about 400 diameters. The photomicrographs were taken by Prof. J. B. Homan of our Department of Medical Art, with the author assisting.

FIG. 1. Silver impregnation following fixation in alcohol-chloroform-acetic acid. The heavy black cables are the nerve fibers, or neurones, which are somewhat shrunken but of fairly regular caliber. There is a paucity of detail in the myelin sheath, the funnels are partially shown and the Schwann cells, with their processes, are easily recognized.

FIG. 2. Silver impregnation following fixation in neutral formalin. The neurones show irregular bulging and are sometimes much shrunken; there is a very irregular fixation. The details of the myelin sheath are somewhat increased, as compared with the preceding picture. Note the beaded nerve fiber at the left of the field.

FIG. 3. Silver impregnation following fixation in Zenker's fluid. The nerve fibers are more uniformly fixed and more contracted. There is a wealth of detail in the myelin sheath, caused either by coagulation of the lipins or by some chemical combination between the salts of the fixative and the silver salts.

FIG. 4. Silver impregnation following fixation in Bouin's fluid. Here the nerve fibers are difficult to follow in their course; they show areas of apparent dissolution, one of which is at the right of the field. The details of the myelin sheath are not well brought out, while those of the longitudinal fibers of the endoneurium are excellently demonstrated.



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Effect of Fixation on Silver Impregnation

SILVER IMPREGNATION OF GLIA AND NERVE FIBERS IN PARAFFIN SECTIONS AFTER FORMALIN FIXATION *

HELENOR CAMPBELL WILDER

(From the Army Medical Museum, Washington, D. C.)

Silver impregnations of nerve fibers and glia comprise a formidable number of long and tedious processes. Many of these methods give beautiful results, but, as they require special fixatives or lengthy and tedious pretreatment, they are impracticable for use in the majority of laboratories where formalin is the routine fixative. Foot [1, 2] has obtained satisfactory glial impregnation in frozen sections after fixation in neutral formalin and, with his work as a starting point, I have endeavored to devise a method that would be applicable to paraffin sections as well.

Using the silver diammino hydroxid recommended by Foot,[2, 3, 4] as a result of his tests of the work of Kubie and Davidson [5] and his formol-sodium carbonate reducer, I experimented with paraffin sections after formalin fixation. The experiments included application on the slide, before impregnation, of reagents advocated as sensitizers of glia to silver. Ammonium bromid,[6] ammonium hydroxid,[7] hydrobromic acid,[7] pyridin,[3] Carnoy's fluid,[8] and other reagents that have been of value in neurological methods were tried without success. Del Río-Hortega's [9] results with formalin-uranium nitrate fixation preparatory to silver impregnation of frozen sections led me to precede silver impregnation of formalin-fixed paraffin sections by treatment with uranium nitrate. A differential reaction to silver was immediately apparent. There was no precipitate deposited on the slide and the sections showed argentation of the fibrillar elements; nerve fibers, fibrillar astrocytes, reticulum and collagen were impregnated. After uranium nitrate, silver staining was so intense that it became necessary to use the silver diammino hydroxid at room temperature rather than at 50° C, and to reduce the time from 30 minutes to 20 seconds. Uranium nitrate in 1 per cent solution proved most satisfactory, but exposure of more than 5 seconds inhibited impregnation. The other nitrates were of no value.

* Received for publication July 1, 1932.

The previous experiments were repeated, introducing the additional step of uranium nitrate, because sharper definition of nerve fibers, more complete staining of glial elements, a clearer background, and an elimination of reticulum and collagen were desirable. Hydrobromic acid accomplished the desired results with the greatest consistency and proved of especial importance as a differentiating factor. Substitution of uranium nitrate for the sodium carbonate of Foot's reducer further accentuated the glial fibers. The gold toning of Foot's revised Variant 3 [4] (1:500 gold chloride, 10 minutes; formalin 5 cc., oxalic acid 0.5 gm., water 100 cc., 10 minutes; 5 per cent sodium thiosulphate, 10 minutes; wash in tap water after each step) tended to clear the background and, where glial fibers showed granular deposits of silver, to give them a more solid and fibrillar appearance. However, the improvement was not consistent and, as it failed to differentiate glia from nerve fibers, it is not included as an essential feature of the stain.

All experiments were carried out on material that had been fixed in unneutralized formalin from two to twenty-four hours after death or removal.

Technique

Fixation and Embedding: Fix tissues in 10 per cent formalin, wash in tap water, dehydrate in alcohol, clear in chloroform, and embed in paraffin.

Bromuration: Pass paraffin sections through xylol and graded alcohols, rinse in distilled water, and place in 34 per cent hydrobromic acid for 30 minutes.

Sensitization: Wash in distilled water 10 to 20 seconds and flood the slide with 1 per cent uranium nitrate (sodium free) for 5 seconds or less.

Impregnation: Wash in distilled water 10 to 20 seconds and place for 20 seconds in silver diammino hydroxid:

To 5 cc. of 10.2 per cent silver nitrate add ammonium hydroxid drop by drop until the precipitate which forms is dissolved. Add 5 cc. of 3.1 per cent sodium hydroxid and just dissolve the resulting precipitate with a few drops of ammonium hydroxid. Make the solution up to 50 cc. with distilled water.

Reduction: Wash in distilled water 2 seconds and agitate each

slide separately in the following reducing solution until it ceases to give off a brown cloud:

Distilled water 50 cc., 40 per cent neutral formalin (neutralized with magnesium carbonate) 0.5 cc., 1 per cent uranium nitrate 1.5 cc.

Counterstaining and Mounting: Wash in distilled water, counterstain with eosin, dehydrate in alcohol, clear in xylol and mount in Canada balsam. Argentation frequently allows hematoxylin to be used as a nuclear stain, but bluing must take place in tap water, as ammonia dissolves the silver.

Distilled water is used in the preparation of all solutions. The uranium nitrate solution and the 10.2 per cent silver nitrate keep indefinitely and the silver diammino hydroxid keeps for a week or more in amber, glass-stoppered bottles. The impregnating and reducing solutions retain their activity in Coplin jars for two days. The hydrobromic acid may be kept in a Coplin jar and used repeatedly for an indefinite time. It is important that the ammonium hydroxid be kept in a well stoppered bottle.

Results

Ganglion cells, nerve fibers, glia cells and their processes are black. Tissues fixed twenty-four hours after death or removal show excellent impregnation of nerve fibers and fibrous astrocytes, but the processes of protoplasmic astrocytes and oligondendroglia cannot be demonstrated when more than six hours have elapsed before fixation. In fresh fixed tissue all the fibers are sharply defined, but tend to become granular when fixation is less prompt. Although differentiation between nerve fibers and glia must be on a morphological basis, the method has the advantage of being quick, simple, and applicable to paraffin sections of formalin-fixed tissue.

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DESCRIPTION OF PLATE

PLATE 123

FIG. 1. Fibrous astrocytes with pedicles to a blood vessel in a medulla fixed twenty-four hours after death. × 600.

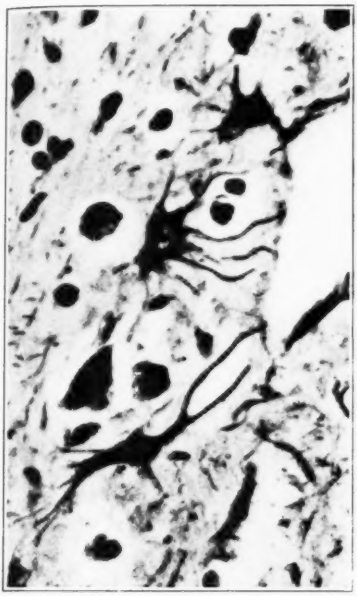
FIG. 2. Large astrocytes around a vessel in cerebral arteriosclerosis. The tissue was fixed twenty-four hours after death. × 475.

FIG. 3. Oligodendroglia in a cerebral cortex fixed six hours after death. × 600.

FIG. 4. Fibrous astocytoma fixed two hours after death. × 600.

FIG. 5. Fibrous astrocytes in a spinal cord fixed twenty-four hours after death. Nerve fibers appear in cross-section. × 475.

FIG. 6. Bipolar cells in a spongioblastoma multiforme fixed ten hours after death. × 475.



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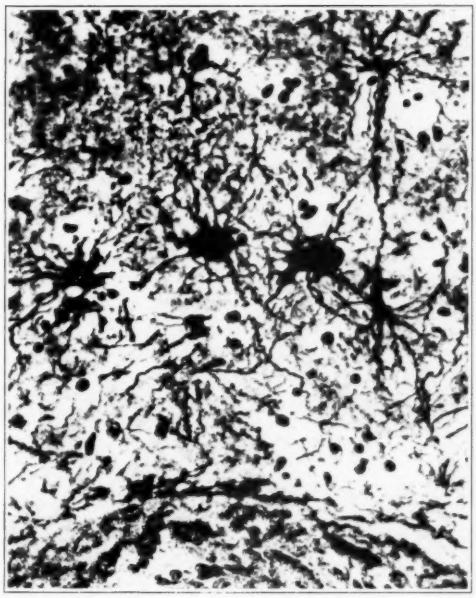

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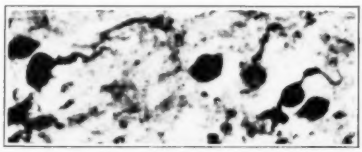

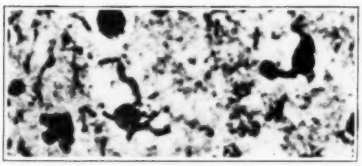

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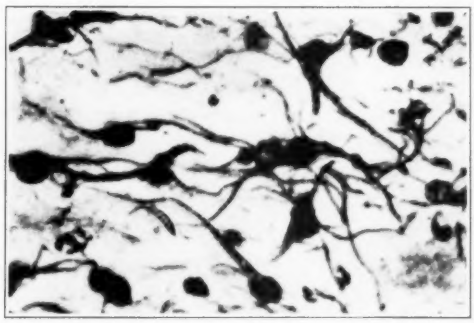

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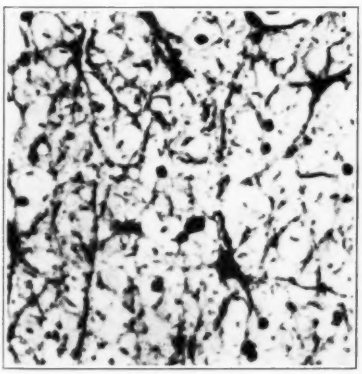

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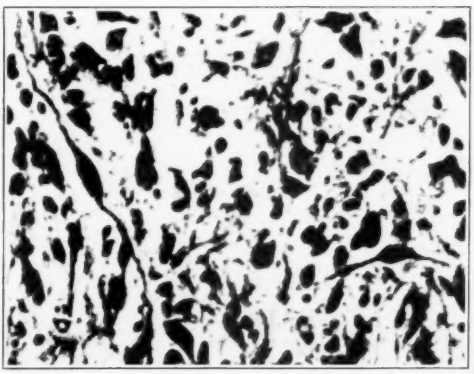

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Wilder

Silver Impregnation of Glia and Nerve Fibers

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